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# THE LIPOSOME AS A MODEL MEMBRANE SYSTEM IN QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSAR)

by

©

GURUPADAPPA BETAGERI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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EDMONTON, ALBERTA

SPRING 1988

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J. A. Rogers
Supervisor

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TO THE MEMORY
OF MY SON
CHIRAG

# ABSTRACT

The partitioning of various analogues of pre-prenalterol (beta blocker drugs) and 2-nitroimidazoles was measured in liposomes and the n-octanol-aqueous phase system and the relevance of each as a model distribution system in quantitative structure-activity relationships was evaluated. Temperature-dependent and solution calorimetry thermodynamics of partitioning revealed major differences in the partitioning of solutes in the two systems. Generally, enthalpies and entropies of partitioning of the beta blockers were large and positive in the n-octanol-buffer system and in fluid-state liposomes and small and negative in gel-state liposomes. The partitioning of some beta blockers could not be detected in the oil-water system or in the gel-state liposomes but all drugs could be measured in fluid-state liposomes. Cholesterol addition to lecithin liposomes decreased partitioning whereas negatively-charged liposomes increased beta blocker and decreased 2-nitroimidazole partitioning. Similarities in the partitioning of propranolol in erythrocytes, erythrocyte ghost cell and liposomes were observed although considerably larger values were obtained in the erythrocytes containing their full protein complement. Protection against erythrocyte hemolysis by propranolol may be attributed to structural changes in the membrane caused by the drug due to its substantial partitioning. Detailed studies of the interactions between unilamellar vesicles and beta blockers using $^{1}$H-NMR demonstrated a correlation between the partition coefficients and the ability to displace praseodymium cation from the bilayers which enabled differentiation between nonselective and cardioselective beta blockers by physicochemical means. Correlations between partitioning of beta blockers in the n-octanol-buffer system, liposomes of various compositions and their pharmacokinetic parameters or corneal penetration were best in liposomes but particularly in negatively-charged liposomes. Cholesterol containing neutral liposomes yielded the best correlations with corneal penetration values. Neutral lecithin liposomes correlated best with pharmacokinetic parameters of the 2-nitroimidazoles. Predictions of pharmacokinetic parameter or corneal penetration were always better in the liposome system with significant improvements by varying the liposome composition than in the n-octanol-buffer system indicating the potential of liposomes to provide greater selectivity in a series of compounds with respect to their partitioning and transport behaviors in biological membranes.

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# List of Abbreviations

| | |
|---|---|
| ABL | Acebutolol hydrochloride |
| ALD | Acute $LD_{50}$ |
| APL | Alprenolol hydrochloride |
| app | Apparent |
| ATL | Atenolol |
| BPL | Bupranolol hydrochloride |
| CHOL | Cholesterol |
| DCP | Dicetylphosphate |
| DMPC | Dimiristoylphosphatidylcholine |
| DPPC | Dipalmitoylphosphatidylcholine |
| DPPS | Dipalmitoylphosphatidylserine |
| Eu | Europium |
| $K_{BC}$ | True red blood cell partition coefficient |
| $K_p$ | Partition coefficient of drug between plasma protein and plasma water |
| Ln | Lanthanide |
| LUV | Large Unilamellar Vesicles |
| MLV | Multilamellar Vesicles |
| MPL | Metoprolol hydrochloride |
| NDL | Nadolol |
| NMR | Nuclear Magnetic Resonance |
| OB | Oral Bioavailability |
| OPL | Oxprenolol hydrochloride |
| PA | Phosphatidic acid |
| PC | Phosphatidylcholine |
| PCR | Plasma Clearance Rate |

| | |
|---|---|
| PDL | Pindolol |
| PP | Peak plasma concentration |
| PPL | Propranolol hydrochloride |
| Pr | Praseodymium |
| PS | Phosphatidylserine |
| $P_T$ | Permeability coefficient across the intact excised rabbit cornea |
| QSAR | Quantitative Structure-Activity Relationships |
| r | Ratio of the fraction of drug nonrenally and renally eliminated |
| $r_A$ | Ratio of the fraction of drug bound and unbound to albumin |
| $r_G$ | Ratio of the fraction of drug bound and unbound to $\alpha_1$-acid glycoprotein |
| R.S.D. | Relative standard deviation |
| $r_T$ | Ratio of the fraction of drug bound and unbound to tissue |
| S.D. | Standard deviation |
| SUV | Small Unilamellar Vesicles |
| Tc | Phase transition temperature |
| TPL | Toliprolol hydrochloride |
| UDU | Unchanged Drug in Urine |
| UV | Ultraviolet |
| Vuss | Steady state volume of distribution referenced to the unbound drug in plasma |

# I. INTRODUCTION

The design of drugs having optimal activity requires an understanding of the relationship between the chemical structure and physicochemical properties of drugs as it pertains to the interaction of drugs with biological membranes. An obstacle in understanding such interactions is the complexity of membranes. Experimentally, real membrane interactions are difficult to study. Thus, an alternative approach of studying model systems which can mimic such reactions has become common practice. The use of model systems has enabled the description of both the qualitative and quantitative behavior of many complex interactions.

The relationships between structure and activity (SAR) have routinely been described in qualitative terms for some time. But in 1963 Hansch et al., (1) introduced the notion of structure-activity relationships in quantitative terms (QSAR). Since then the rapid developments in QSAR have occurred and have been reviewed (2-7) and now there is a substantial body of knowledge with respect to such studies.

Meyer (8) and Overton (9) showed that the narcotic activity of many simple organic compounds paralleled their oil/water partition coefficients and they initiated the use of such measurements as a means for defining relative hydrophobicity or lipophilicity of biologically-active organic compounds. However, the interest that was created in these early studies gradually subsided as it was felt that such a general parameter as a partition coefficient was of little value for rationalizing highly specific drug activity. But again in the early 1950s, Collander (10) generated new interest in the relationship between oil/water partition coefficients and activity by demonstrating correlations between the rate of penetration of plant cell membranes by a wide variety of organic compounds and their partition coefficients.

The partition coefficient (K) first defined by Berthelot et al. (11) is, the equilibrium ratio of concentrations existing as a single neutral species of a compound in a nonaqueous phase $[D]_n$ and aqueous $[D]_a$ phase respectively. Thus,

$$K = \frac{[D]_n}{[D]_a} \tag{1}$$

It is also recognized that K of an electrolyte molecule may be a pH-dependent property. The physical property of the molecule which governs its partitioning into a nonaqueous solvent from an aqueous medium is referred to as its hydrophobicity or lipophilicity.

The concept of the hydrophobic bond, which came from Frank and Evans (12), arose from the evidence that nonpolar structures in water are surrounded by loosely-structured water molecules. The importance of the hydrophobic interaction of nonpolar compounds with biochemical systems was examined critically by Kauzmann (13). The hydrophobic bond is actually not a bond formation at all, but rather the tendency of hydrophobic molecules or hydrophobic parts of molecules to be squeezed out of water because these are not readily accommodated in the highly hydrogen-bonded structure of water. Thus, attraction between similar hydrophobic species is accentuated in water resulting in what is referred to as a hydrophobic interaction or hydrophobic bond. The bond involves Van der Waals forces, hydrogen bonding of water molecules in a three-dimensional structure, and other types of intermolecular interactions. The hydrophobic interaction is favored thermodynamically because of the increased disorder or entropy of water molecules that accompanies the association of the nonpolar molecules when they become squeezed out of water (14).

Although the K of a molecule can be used as a measure of its hydrophobicity it is more common to use the relative hydrophobicities of its functional groups. In this case knowing the relative hydrophobicity of the substituents of a parent compound can often be sufficient for structure-activity studies. In fact, one sometimes finds that only substituents in certain positions interact hydrophobically with a given system (15, 16). In order to determine the contribution of the relative hydrophobicity of substituents and in this way separate hydrophobic character from electronic and steric effects of the substituents, the parameter $\pi$ was defined by Hansch and coworkers (17, 18) as:

$$\Pi_x = \log K_x - \log K_H \qquad (2)$$

where $K_x$ and $K_H$ is the partition coefficient of the derivative and the parent compound, respectively. A positive $\pi$ value means that in comparison to H, the substituent x, favors the organic phase. A negative $\pi$ value is indicative of its hydrophilic character relative to H.

The property of hydrophobicity is measured in terms of the relative affinity of a molecule for a nonpolar phase to that of water. Meyer and Overton (8, 9) chose olive oil/water as a model system to measure hydrophobicities or alternatively, partition coefficients. Later in 1935, Meyer and Hemmi (19) re-evaluated various solvent systems and concluded that an alcohol such as oleyl alcohol would be a better model of fatty phases. Collander (10), in the 1950s, also favored the alcohols and considered octanol as a possible reference solvent. Substantial studies (20-23) have confirmed that semipolar hydrogen-bonding solvents are best suited to model the partitioning of lipophilic substances. It had been suggested by others(24, 25) that since hydrophobic part of biological membranes consist of lipid bilayers, a hydrocarbon solvent might be better suited to model the partitioning of solutes in the hydrocarbon-like regions of membranes. Furthermore, a hydrocarbon solvent reference system would be theoretically simpler to describe since there would be minimal interaction with the partitioning solute. However, such solvents suffer from the disadvantage that most drugs are essentially insoluble in them, with the result that partition coefficients can not be measured with sufficient accuracy to be useful. When a polar molecule partitioning into semipolar organic solvents such as the alcohols it carries attached water molecules with it, giving rise to several different species of solute, thus complicating any interpretation of molecular interactions from the partition coefficients (20). It has been argued that a bulk hydrocarbon liquid is not a good model of the lipid bilayer (26, 27), although n-octanol is probably the most widely used solvent for partitioning studies (18). Octanol is a good solvent for compounds because of its semipolar nature but also because of the solubility of water in it (2.3 M at saturation). Water-saturated n-octanol is sufficiently polar that dissolved molecules tend to associate with solvent rather than each other and it has a regular structure which is not changed by the addition of solute (20). n-Octanol is chemically stable, commercially available, nonvolatile and it does not absorb ultraviolet light. Each of these characteristics is of practical importance.

Collander (28) established a relationship between the partition coefficients of solutes in two different phase systems $K_1$ and $K_2$, as

$$\log K_1 = a \log K_2 + b \qquad (3)$$

where the constants 'a' and 'b' of equation (3) have been used to describe the relative hydrophobicities of various two phase systems with respect to several classes of compounds.

In QSAR studies n-octanol-water system has been widely used. However, it is not clear whether it is the ideal solvent system for modelling all the interactions of organic compounds with biological systems. Martin (29) has emphasized that various absorption phenomena involving the passage of drugs across biological membranes are not well modeled by octanol-water partition coefficients.

Phospholipid vesicles (liposomes) have been investigated as a model system to study solute distribution into membranes since these closely resemble the ordered structural features of biological membranes (30-32). The properties of liposomes, such as high permeability to water and electrolytes, osmotic activity (33), and the impermeability of charged liposomes to oppositely-charged ions (34) favor these systems in mimicking biological membranes.

There is now a considerable body of knowledge regarding the structure and properties of liposomes. However, there has not been any systematic study to evaluate the liposome as a model for the distribution of drugs in the same fashion as carried out with bulk oil-water systems. Depending on the nature of the solute, correlation with biological activity may occur above or below the phospholoipid phase transition temperature (Tc) which clearly suggests that structure and order within the phospholipid bilayers is an important determinant of the extent to which a solute is able to interact with the membrane (35). The liposome system has been shown to discriminate against branched solutes more than does a bulk solvent of semipolar character (30). Furthermore, liposome/saline hydrophobicities were found to correlate with the biological activities of chloramphenicol congenors much better than the n-octanol-water system (36).

This thesis is aimed at developing a partitioning model using phospholipid vesicles (liposomes) to improve the correlation with biological properties of derivatives of pre-prenalterol (beta blockers) and 2-nitroimidazoles with the following objectives:

1. To compare the partitioning of a series of beta adrenoreceptor blockers and nitroimidazoles in the n-octanol-water system and in a liposome system.
2. To identify differences between two partitioning model systems using a thermodynamic approach.
3. To study the effects on partition coefficients of liposome compositions versus the n-octanol-water system and to compare correlations between pharmacokinetic/biological activity parameters and partition coefficient in both model systems.
4. To identify specific drug-liposome interactions: i)Interaction of cardioselective and nonselective beta blockers with unilamellar DMPC liposomes using NMR. ii)Evaluation of the role of specific interactions which occur following partitioning of propranolol in erythrocytes, erythrocyte ghosts, liposomes and the

n-octanol-water system and the relationship of such interactions to the action of this drug as a membrane stabilizer.

5. To show how differences in the structures of liposomes and the n-octanol-water system may account for the prediction of biological properties from partition coefficients.

# II. BACKGROUND

## A. QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSAR)

QSAR provides a means of ranking or predicting biological effects of chemical entities based on some function related to structure. For each compound used in a structure-activity model, two kinds of quantitative information are required, namely, biological activity and molecular properties. Albert (37) has been foremost in pointing out that there might be a quantitative relationship between biological potency and chemical properties in a series of drugs. Hansch and coworkers (38, 39) were the first to propose the model for QSAR. They also proposed that the observed relationship between lipophilicity and biological potency of solutes be incorporated into the useful equations of linear-free energy relationships (LFER) to provide a general model for QSAR in the biological context (40). The success of Hansch in demonstrating that free energy correlations can be successfully applied to biological processes has prompted some workers to reexamine the Hansch equation in greater detail. Using the principles of theoretical pharmacology or pharmacokinetics (41, 42), some have attempted to develop an improved theoretical model to accommodate some of the complex relationships observed between biological activity and chemical structure or properties of compounds, and to broaden the scope of the Hansch equation. Excellent discussions of these models have been reviewed by Martin (6, 43) and Kubinyi (7). In spite of this, the free energy model of Hansch has still been the most widely used because of its broad applicability and its simplicity.

## Quantitative Linear Free-Energy Relationships for Chemical Reactions of Organic Compounds:

### Univariant Relationships: Electronic Effects of Substituents on Benzene

Hammett (44) studied the structure-activity relationships of the effect of sterically remote substituents on the equilibrium or rate constants of organic reactions. Substituent effects in such reactions are electronic in nature. By this he meant the substituted effect is due to changes in the electrostatic forces at the reaction center. Electronic effects are subdivided into inductive-field and resonance effects.

The Hammett equation can be expressed as

$$\log\frac{k}{k_0} = \rho\sigma \tag{4}$$

where 'k' refers to the rate or equilibrium constant for the reaction of the substituted compound, '$k_o$' corresponds to that of the reference compound. The proportionality constant $\rho$, gives an indication of the sensitivity of the reaction to electronic effects.

Hundreds of structure-activity relationships (45) have been observed to fit Eq. (4) and both equilibrium and rate constants have been found to obey this relationship. Thus, sigma constants appear to be reliable descriptors of the electronic influence of different substituents.

Properties correlated with sigma ( $\sigma$ ) constants are usually described as being additive, which means that multiple substituents exert an influence equal to the sum of the individual substituents, and constitutive which means that the effect of a substituent may differ depending on the molecule to which it is attached or its environment. A simple illustration of the constitutive nature of sigma constants is when the same substituent has a different sigma value when substituted at the meta or para positions.

It should be noted that not all series produce a single linear relationship between sigma and log k (46). A nonlinear relationship is interpreted to mean that a change in the predominant mechanism or a change in the rate-limiting step in the reaction has occured.

These relationships are extrathermodynamic because although the relationships between the reactions are stated in thermodynamic terms ($\Delta G$ or log k values), there is no thermodynamic principle which says that these relationships should be true.

The Hammett relationship provides a quantitative equation which allows one to compare the relative sensitivity of reactions to electronic or polar effects. Conversely, it provides a method to examine the relative electronic effect of different substituents.

## Multivariant Relationships: Steric and Electronic Effects Operating Simultaneously

Taft extended the Hammett equation to include the steric effects of substituents and also to include the effect of substituents of nonaromatic parent compounds (47). The steric effect of a substituent was assigned the symbol $E_s$, and the electronic effect of a substituent on an aromatic parent, $\sigma^*$. The Taft steric parameter $E_s$ is expressed as

$$E_s = \log \left[ \frac{k}{k_0} \right]_a \tag{5}$$

where 'k' and '$k_o$' refer to the substituted and parent compound respectively. The subscript 'a' denotes acid catalysis.

Taft also described an equilibrium state in which more than one effect of substitution was considered in the prediction of reaction rates or equilibria. Thus,

$$\log \frac{k}{k_0} = \rho^* \sigma^* + \delta E_s \tag{6}$$

either $\sigma^*$, $E_s$ or both may contribute to the observed differences in the rate or equilibrium constants.

## B. DESCRIPTION OF THE EXTRATHERMODYNAMIC METHOD

A basic premise of the extrathermodynamic concept is that the relative biological effects of members of a set of analogs are attributed to influence the substituent has on the chemical and physical properties of the molecule. The behaviors which substituents impart on a molecule usually is tested using model compounds and reactions. For instance, the effect of a substituent on the distribution of a compound between water and a nonaqueous biological phase is determined from its effect on the molecule as it reflects its oil-water partition coefficient (K) of a compound, and also on its electronic effect as described by $\sigma$ or $\sigma^*$.

Application of the extrathermodynamic method is not limited to the retrospective study of QSAR within a series. The assumption of the relationship between chemical properties and biological activity can be practically used in the design of a series of analogs to follow up a lead. The initial series contains a maximum variation in the properties of usual importance, a substitution pattern in which these properties are not correlated, and the minimum number of compounds. If the analysis is performed using the model-based equations, fits to the equations can provide predictions with respect to the molecular pharmacology of the drug-receptor interaction. In certain cases one can distinguish if the ion or the neutral form is responsible for the observed activity. It is an assumption of this method, that all analogs of the series react with the same receptor in an identical fashion.

## Early Studies of the Biololgical Properties of Organic Compounds

Failure of the Hammett sigma value to predict the relative potency of some plant-growth inhibitors, prompted Hansch et. al. (15) to identify a more relevant physical property, namely, the partition coefficient, K, and they demonstrated that log K was an additive-constitutive property (17, 48). Hence, the change in log K due to a substituent may be readily predicted. Actually, it is well established that each successive addition of a methylene group into a molecule increases log K by a fairly constant amount. However, no one had previously emphasized the utility and generality of this observation in QSAR studies.

A substituent produces three different major changes in the physical properties of a molecule: electronic, steric and hydrophobic. Similarly it was postulated that a corresponding effect of substituents on the biological properties of a molecule may also occur (15) on the assumption that the electronic, steric and hydrophobic influences of substituents on drug potency (1/C) are independently additive, the following relationship was developed:

$$\log\frac{1}{C} = a\log K + b\,E_s + \rho\sigma + d \tag{7}$$

The validity of equation (7) (the Hansch equation) is usually based on the correlation coefficient from multiple regression analysis. The biological activity of approximately 135 such relationships has been reported by Hansch and Dunn (49).

### Parabolic Relationship Between Potency and log K

The validity of the linearity of the Hansch equation was discovered to be limited to a certain range of concentrations. The relationship is linear and positive at relatively low log K values, then potency reaches a maximum then gradually decreases with further increase in log K. Hansch and co-workers (15) further proposed a parabolic relationship given by

$$\log \frac{1}{C} = a \log K - b \log K^2 + cE_s + \rho\sigma + d \qquad (8)$$

and over 150 examples of parabolic relationships with log K have been reported by Hansch and Clayton (50).

## C. PARTITION COEFFICIENTS

The partition coefficient is the ratio of concentrations at equilibrium of a solute distributed between two immiscible phases; the concentration in the more lipophilic phase is, by convention, the numerator. The term 'immiscible' does not preclude the two phases having partial miscibility. For example, water-saturated 1-octanol contains about 27% mole of water (51). The term "partition coefficient" is restricted to defining the concentration ratio of the same molecular species, as was first pointed out by Nernst (52). The terms "distribution coefficient" or "apparent partition coefficient" apply to the ratio of total concentrations, of ionized and associated species.

The justification for the use of log K is primarily that this is a free-energy related term, from the Van't Hoff isotherm

$$\Delta G = -RT \ln K \tag{9}$$

where ΔG represents the free energy change during the transfer of the solute from water to nonaqueous phase.

### Correlation Between log K Values in Different Solvents

Since hydrophobicity depends so heavily on the structure of water it is not surprising to discover that there is a linear correlation between the log K values of compounds between various solvent-water systems (10, 53). Log K of compounds partitioned between octanol-water and between oleyl alcohol, primary butanols, secondary and tertiary pentanols, cyclohexanol and water have yielded excellent correlations (r=0.99).

For solvents which do not contain a polar group (benzene, cyclohexane) the log K values of solutes which are "hydrogen-bond donors" and "hydrogen-bond acceptors" are correlated by different equations (53). Within each type of compound however, there is often an almost perfect correlation between the n-octanol-water log K value and the other solvent and water.

Hansch and Dunn (49) reported that for a series of phenols the difference between the octanol-water log K and that between cyclohexane and water is quantitatively explained by hydrogen bonding. Seiler (54) studied this problem by means of regression analysis of the difference between the n-octanol-water and cyclohexane-water log K values of 230 compounds and found that this difference is totally explained by the substituents present in the molecules. Accordingly, a new additive constitutive substituent constant $I_H$ was defined as follows

$$I_H = \log K_{octanol} - \log K_{cyclohexane} - 0.16 \qquad (10)$$

The values of $I_H$ for a substituent is approximately proportional to its hydrogen bonding ability. Hydrogen-bond formers have a relatively higher log K in octanol than in cyclohexane. The $I_H$ parameters may be used as a variable in the structure-activity analysis or as a correction to the octanol-water log K.

## Hydrophobic Substituent Constant

The partition coefficient (log K) is used as a measure of the hydrophobicity of a whole molecule, while working with a set of derivatives of a parent compound in which a large portion of the structure remains constant. In such a case, knowing the relative hydrophobicity of substituents can be sufficient for correlation analysis. The relative hydrophobicity of substituents, the parameter ($\pi$) has been defined (17, 18)

$$\Pi_x = \log K_{mx} - \log K_{mH} \tag{11}$$

where $K_{mx}$ is the partition coefficient of a derivative and $K_{mH}$ is that of parent compound. In general, for substituents on an aromatic ring, $\pi$ values are constant whether the parent is benzene, benzoic acid or phenylacetic acid.

## Measuring the Partition Coefficient

The measurement of partition coefficients is in principle very simple. One merely equilibrates the solute between an aqueous solution and n-octanol and measures the concentration of solute in the two phases. In practice one must be certain that pure substances are used for solute, solvents and buffers, equilibrium has been obtained, and that the final result is the equilibrium value of the nonprotonated monomeric species between the two phases. The stability of the compound should be verified under the conditions chosen. Any ionizable substance may be used as a buffer provided that it does not interact with the solute or interfere with the analytical method and that it maintains the pH. Albert and Serjeant (55) listed several possible choices.

In situations of ionization of the compound only the unionized form partitions then only an apparent K' can be determined. However, it is possible to calculate the corresponding 'true' or ion-corrected partition coefficient

$$K'_{app} = \frac{C_{oil\ (undissociated)}}{C_{water\ (undissociated)} + C_{water\ (dissociated)}} \qquad (12)$$

$$K_{ion\text{-}corrected} = \frac{K_{app}}{(1 - \alpha)} \qquad (13)$$

$$\alpha = \frac{1}{1 + \text{antilog}\,(pH - pK_a)} \qquad (14)$$

where $\alpha$ is the fraction ionized and when equation (13) is used the exact $pK_a$ must be known.

## D. THERMODYNAMICS OF PARTITIONING

The Partition coefficient is a free energy related term as shown in the following equations (58).

$$\begin{aligned} \ln K &= \frac{-\Delta G^0_{w \to L}}{R\,T} \\ &= \frac{-\Delta H^0_{w \to L}}{R\,T} + \frac{\Delta S^0_{w \to L}}{R} \end{aligned} \qquad (15)$$

subscripts L and w refer to the lipid phase and aqueous phase, respectively. The superscript 'o's denote that the thermodynamic quantities are measured at standard state. The corresponding changes in standard partial molar free energy, enthalpy and entropy, respectively on transferring solute from water to the lipid phase, are given by

$$\Delta G^0_{w \to L} = G^0_L - G^0_w \qquad (16)$$

$$\Delta H^0_{w \to L} = H^0_L - H^0_w \qquad (17)$$

$$\Delta S^0_{w \to L} = S^0_L - S^0_w \qquad (18)$$

where $G^o_W$, $G^o_L$, etc., are the standard partial molar state functions of solute in each phase.

## Partial Molar Free Energies of Partition

Using equation (15) and K values, one can calculate partial molar free energies of partition. $\Delta G^o_{W \to L}$ has the physical meaning of the change in free energy on transferring one mole of solute from a hypothetical aqueous solution to a hypothetical solution in lipid, each solution containing solute at a concentration of 1 molal and having the physical properties of an infinitely dilute solution.

## Partial Molar Enthalpies of Partition

The temperature dependence of partitioning can be used to obtain the enthalpy of the process based on the relationship (59) $(\delta H^0_{w \to L} / \delta T)_p = T(\delta S^0_{w \to L} / \delta T)_p$ (19)

and the assumption is made that $\Delta H^o_{W \to L}$ and $\Delta S^o_{W \to L}$ are approximately independent of temperature (60). $\Delta H^o_{W \to L}$ has the physical meaning of the change in enthalpy when one mole of solute is transferred from water to lipid at infinite dilution. Since $\Delta G^o_{W \to L}$ is obtained directly from K, while $\Delta H^o_{W \to L}$, as obtained from the slope of log K against 1/T, is more sensitive to experimental errors than $\Delta G^o_{W \to L}$.

**Partial Molar Entropies of Partition**

The fundamental thermodynamic relationship is

$$\Delta G = \Delta H - T \Delta S \tag{20}$$

as applied to the partitioning of a solute, following rearrangement becomes

$$\Delta S^0_{W \to L} = \frac{\Delta H^0_{W \to L} - \Delta G^0_{W \to L}}{T} \tag{21}$$

$\Delta S^o_{W \to L}$ has the physical meaning of the change in entropy when one mole of solute is transferred between two hypothetical mixtures, in each of which the solute has a molal concentration of 1 but has the same physical properties as in an infinitely dilute solution.

**Measurement of Partial Molar Enthalpy using Solution Calorimeter**

Heats of reaction, either endothermic or exothermic, can be determined in many different systems, ranging from simple acid-base reactions to more complicated redox, chelation, hydrolysis, protonation and other reactions. The calorimeter is particularly effective for assays of weak acids and weak bases which do not respond readily to other detection methods. The heat of solution produced when a solid dissolves in a liquid can be readily measured by solution calorimetry. The solution calorimeter consists of a glass dewar reaction chamber with a rotating sample cell, a thermistor probe and a specially designed temperature measuring bridge, all assembled in a compact cabinet. Temperature changes are plotted directly using an accessory strip-chart recorder. The energy change,(Q), in calories is calculated from multiplying the net corrected temperature change, $\Delta Tc$, and the energy equivalent (e), of the calorimeter and its contents accordingly:

$$Q = (\Delta T_c)(e) \tag{22}$$

The change in enthalpy, $\Delta H$, at the mean reaction temperature is given by -Q and sample weight in moles or grams as

$$\Delta H_T = -Q/m \tag{23}$$

where the mean reaction temperature is the temperature at the 0.63R point on the thermogram (61).

## E. LIPOSOMES

### General Description and Properties

In the early 1960s, great emphasis was placed upon the study of the properties of lipid-water systems as model biomembranes. Bangham and others (33) studied these systems from different points of view, aiming to gain insight into biomembrane properties.

Compounds which exhibit amphiphilic properties to the extent that they sustain highly ordered sheet-like micelles or closed membranes in equilibrium with water are the polar lipids such as phosphatidylcholines (PC), phosphatidyl ethanolamines (PE) and phosphatidyl serines, sphingomyelins, cardiolipins, plasmologens, phosphatidic acid (PA) and cerebrosides. When phospholipids are hydrated they undergo a sequence of assemblages, which, step-by-step, reflect the thermodynamic perturbations of increasing oil-oil, water-oil and water-water interaction. The final structures, referred to as liposomes which are layer lattices of alternating, closed bimolecular lipid sheets intercalated by aqueous spaces, persist as equilibrium structures even in the presence of excess of water(56).

Liposomes can be prepared from a variety of lipids and lipid mixtures, with phospholipids being the most commonly used (79). However, vesicles can be prepared from single chain amphiphiles (80), lysophosphatides in the presence of equimolar cholesterol (81) and dicetylphosphate (82). The different types of liposomes are usually referred to by a three letter acronym, e.g. multilamellar vesicles (MLV), small unilamellar vesicles (SUV) under 1000 A° in diameter, and large unilamellar vesicles (LUV) from 1000 A° to 2000 A°.

In many cases liposomes have been prepared from mixtures of different phospholipids. Saturated phospholipids of the same head group but differing by only two methylene units in the acyl chains, will exhibit complete miscibility in all proportions. However, non ideal mixing will occur with evidence of lateral phase separation of the phospholipids when the chain lengths differ by four or more methylene groups (83). Similar results are observed with mixtures of saturated PGs and PCs, both in the presence and absence of calcium (84). In the latter system, calcium does not induce a phase separation when the PG chain lengths are equal or two carbons longer than the PC but does so when the PG chain lengths are two carbons shorter (84). Such calcium induced lateral phase separation has been observed initially in PS/PC mixtures (85) and later with PA/PC mixtures (86) and DPPS/DPPC mixtures (87). Mixtures of PCs and PEs of equal chain lengths also exhibit lateral phase separations in the gel phase in MLV (88) which appear to be accentuated in SUV (89). PE appears to be unique among the phospholipids in not forming closed bilayer vesicles at neutral pH (89), but rather a hexagonal phase structure (90). In fact, vesicles cannot be formed from PE (at neutral pH) if the mole fraction of PE is greater than 70% (91).

In CHOL-PC mixtures the composition of the two monolayers in the bilayer is found to be similar up to 30 mole % of cholesterol (92), but at higher concentrations cholesterol resides preferentially in the inner monolayer. Thus, when using mixtures of lipids to form SUVs, it is possible that the composition of the outer monolayer may not be the same as the composition of the initial mixture. The resulting distribution depends upon the packing requirements of the individual head groups, the acyl side chain composition, the surface charge of the phospholipds, and pH.

The study of the submicroscopic and dynamic properties of these model biomembranes such as fluidity (62), phase transitions and change to other mesomorphic structures was for some time carried out distinctly and separately from the study of more macroscopic properties such as their ion trapping and release characteristics (63). Considerable interest and excitement was created by the possibility of *in vivo* application of liposomes in medicine. Sessa and Weismann (64) and Gregoriadis et al. (65) considered the liposome as a vehicle for replacement therapy in genetic deficiencies of lysosomal enzymes.

1. Encapsulation of Water-Soluble Compounds: The spontaneous rearrangement of anhydrous phospholipids in the presence of water into a hydrated bilayer structure

is accompanied by the entrapment of a portion of the water in an aqueous space between repetitive continuous closed bilayers(lamellae). For water-soluble compounds of relatively small molecular weight, their encapsulation depends upon the relative amounts of lipid and water present in the preparation, the liposome size, and the number of lamellae. Also, in addition to passive entrapment the application of a pH gradient across the bilayer of a liposome has enabled greater concentrations of catecholamines of 12 to 23 fold to be entrapped (66).

2. Incorporation of Lipid Soluble Compounds: Lipophilic compounds can become entrapped in liposome by intercalation with the phospholipids in the bilayer. The mode of interaction of lipophilic compounds with liposomes determines their degree of permeability, as well as the size and stability of the liposomes and is a function of both the composition and amount of the added lipophilic compound. Amphipathic compounds such as detergents below their CMC intercalate into the bilayer increasing the permeability to entrapped compounds, whereas concentrations above the CMC lead to disruption of the bilayer (67, 68). Maximal association of lipophilic compound with liposomes is usually obtained by including the compound in the organic phase during liposome preparation (69).
3. Permeability: The permeability through liposomal bilayers generally increases with decreasing acyl chain

length of phospholipid and the degree of unsaturation of the acyl chains (70). In liposomes composed of single, pure phospholipids the permeability to solutes is relatively low <Tc, exhibits an anomolous increase and decrease at the vicinity of its Tc, and increases further at temperatures above the Tc (71, 72). When the lipid composition of the liposome includes at least 33 mole% CHOL, the permeability is decreased and the anomolous permeability increase in the vicinity of the transition temperature is eliminated (73, 74). The permeability of various molecules in liposomes occurs in the following order: water> small nonelectrolytes> anions> cations(75).

4. Stability: Liposomes can become physically unstable on storage due to either leakage of the encapsulated drug into the suspending medium or to aggregation or fusion of the liposomes to form larger entities. Frokjaer et al. (76) reported that the increase in size and drug leakage on storage of small unilamellar liposomes were dependent upon the nature both of the lipid components and of the encapsulated marker compounds. Drug retention behavior of a range of liposomal compositions under various conditions of storage was cited by Szoka and Papahadjopoulos (77).

The chemical stability of the lipid components of a liposomal system are subject to various degradative processes on storage. Hunt and Tsang (78) showed that the oxidative decomposition of egg lecithin in liposomes can be retarded drastically by addition of $\alpha$-tocopherol to the lipid mixture. Both unsaturated and saturated phospholipids are subject to hydrolysis in aqueous media, resulting initially in the formation of the corresponding lysophospholipid and fatty acid. Frokjaer et al. (76) reported that the hydrolytic degradation of a long, saturated chain lecithin was rapid at both acidic and alkaline pH values.

## Role of the Phospholipid Thermotropic Phase Transition

The importance of the thermotropic phase transition temperature is demonstrated by the evidence that water diffuses into the polar region of the bilayer only >Tc i.e. when the hydrocarbon chains "melt".

Vesicles composed of phospholipids that are at temperatures below the Tc are considered as "solid"; when above Tc they are considered as "fluid" (94). The Tc is a function of acyl chain length, and increases by about 14-17° with every 2-methylene unit increase in chain length. The presence of unsaturated acyl chains, branched chains or those carrying a considerable bulky side group, e.g. cyclopropane rings, produces a considerable decrease in the Tc. In mixed acyl chain phospholipids composed of saturated fatty acids that differ by two methylene units, the isomer that has the longer chain in the sn-1 position of glycerol has the lower Tc of the pair (95). Also the head group of the acidic phospholipid, such as PS, PG or PA can have a considerable influence on the Tc (96). The Tc of these phospholipids can be modulated by interactions with divalent cations or by H ion titration of the head group. Calcium and magnesium ions in the physiological range (0.1 - 10 mM) can increase the Tc of PA, PS and PG (97) and the addition of a hydrogen ion increases the Tc by 17° C in DPPG (98)and by 6-8° in DMPA and DPPA (99).

**Methods of Preparation**

Different methods of preparation are known to yield liposomes of different shapes and sizes. These range from large myelinic structures, to oblong MLVs, to small spherical vesicles. Concurrently, their sizes may vary in dimension from several hundred A° to fractions of a millimeter, so that a whole range of sizes and shapes can coexist. Phospholipids may be dispersed by a variety of techniques to form multilamellar liposomes and also to form unilamellar liposomes. The term "liposome" is frequently used to encompass this entire range of multilamellar or unilamellar structures(93), although SUVs are also referred to as vesicles.

Different methods of liposome preparation are known of which the most important ones are:1)the typical Bangham method (63), 2)the ether evaporation method, 3)the detergent removal method 4)the reverse-phase evaporation vesicle method (REV) (110) and 5)the dehydration-rehydration method

(101). Several variations of these methods have been reviewed (102-108). Size selection of liposomes has been accomplished by sonication (100), gel permeation chromatography (101), extrusion techniques and the application of the french pressure cell (109-111).

## Application of NMR to Study Drug-Liposome Interactions

Over the last decade, the orientation and characteristics of the properties of phospholipids in model membranes have been studied by new spectroscopic probing methods. Many of these techniques have contributed to our present understanding of a membrane. Studies employing NMR techniques have provided some of the more detailed data.

Phospholipid membranes can be examined from two different viewpoints. Firstly, the membrane is considered as a collection of phospholipids with particular macroscopic properties, e.g. the thermodynamic properties, such as phase behavior and transitions. The second viewpoint is a molecular or microscopic one, which examines the structural and motional properties of the various parts of the phospholipid molecule. Both aspects can be studied by NMR methods. Accurate descriptions of the various phospholipids are necessary in order to interpret such complex phenomena as protein-lipid, drug-lipid or ion-lipid interactions (112).

$^{2}$H, $^{13}$C, $^{19}$F, $^{31}$P and $^{1}$H NMR have been the main atoms used in detailed studies. The application of $^{1}$H-NMR to membrane studies has been hindered by two major problems: an ability to obtain resolved spectra from dispersed or natural membranes, and the lack of an easily characterizable physical parameter. High resolution spectra are obtained with small sonicated vesicles, but in dispersed systems these resonances broaden into large featureless signals. Some success has been obtained with intermediate sized vesicles (113). The exact nature of the broadening of the signals in dispersed samples is still not completely understood.

### Lanthanide Ions in Membrane Studies

One of the earliest uses of $Ln^{3+}$ ions to probe biological membranes is their employment as shift reagents in NMR studies of phospholipid bilayer vesicles. In 1971 Bystrov et. al. (114) reported that the external $Eu^{3+}$ ions shift the $^1H$-NMR resonance of the trimethylammonium head group of lecithin molecules in the external layer of a bilayer vesicle to higher field while leaving the corresponding interior resonance unshifted. Increasing the concentration of $Eu^{3+}$ increased the line width of the external trimethylammonium resonance. This behavior indicates a rapid exchange of $Eu^{3+}$ ions interacting with polar head groups on the exterior surface.

Fernandez and Cerbon (115) used the splitting in the proton resonance of the trimethylammonium head group of egg yolk lecithin vesicles induced by $Eu^{3+}$ and $Pr^{3+}$

ions to study the displacement of these ions by the local anaesthetics. They found that the downfield shift produced by $Pr^{3+}$ ions was larger than the upfield shift caused by $Eu^{3+}$ ions. Hydrophobic interaction with the lipid portion of the vesicle appears to neutralize the lecithin phosphate moieties as sites of $Ln^{3+}$ ion binding(115).

## F. THE LIPOSOME AS A MODEL PARTITIONING SYSTEM IN QSAR

Liposomes have been used previously as model partitioning systems and the contributions of substituents to the thermodynamics of partitioning of alcohols, phenols, phenothiazines and beta blockers in DMPC liposomes have been studied in some detail (30, 31, 116-119). Usually, a significant decrease in the partition coefficient 'K', is observed upon cooling the liposomes below the Tc of the phospholipid. The particular structures and hydrophobicities of drug molecules contribute to their distribution in liposomes. Drugs can be located either in the lipid bilayers or associated with the polar head groups at the surface of the bilayers. The partitioning of phenothiazines in DPPC liposomes has also been studied (120). Wright and Bindslev (35) demonstrated that permeations of solutes across the toad bladder were closely related to their liposome distribution data when the liposomes were below the Tc. In contrast, the liposome partitioning of a series of chloramphenicols in DMPC liposomes has been reported to yield a better correlation with their biological activities above the Tc in comparison to the n-octanol-water system (36).

## Selection of Model Compounds

A series of homologous compounds is desirable in studies of quantitative structure-activity relationships (QSAR) to make rational drug design possible. It is a prerequisite that data on the physicochemical and biological properties exist for a sufficient number of these compounds. The QSAR established for these reference compounds could then provide a basis for the prediction of the *in vivo* performance of new compounds within the parameter range covered. Two series of compounds were selected for the present study on the basis of the following criteria:

1. selection of a series of compounds having a common parent structure
2. a wide range of hydrophobicities
3. available information concerning their biological properties
4. reasonable chemical stability
5. known methods of analysis
6. pharmacological importance

## Beta Adrenoreceptor Blocking Agents

In 1948 Ahlquist (121) first proposed the classification of adrenergic receptors into two subtypes, α and β and further subdivisions of receptors has been proposed (122-124). Beta adrenergic blocking agents were developed as agents that might protect the heart from sympathetic drive and, therefore, prevent ischemia in patients already suffering from angina pectoris (125). Clinically, beta blockers have found wide use in a number of diverse indications, not all of which depend on direct beta adrenergic blockade (126-129).

Beta-blocking drugs can be divided into those that are nonselective and those that have a selective action on one type of beta receptor, beta-1 or beta-2, and further into drugs that, in addition, possess alpha receptor blocking properties(130). Beta blockers appear only to occupy the receptors, while they prevent continuing function by competitively inhibiting access of agonists (131). According to the structure of the side chain, inhibitors are divided into two main classes, namely, the aryl- or heteroaryl-ethanolamines and the aryl- or heteroaryl-oxypropanolamines (132). It is not essential for beta-blocking activity that the side chain can be attached directly to a benzene nucleus; The isopropyl-substituted ethanolamine maintains activity when attached to various heteroacyl structures such as naphthyl (133), benzodioxanyl (134) benzodioxinyl (135), benzofuranyl (136) and indole rings (137). With pindolol and timolol (138), considerable potency is associated with a heterocyclic ring.

## Structure-Activity Considerations

Beta-blockers vary widely in their lipid solubility. The affinity constant of eight beta-blockers for microsomal cytochrome P-450, causing type-I spectral shifts have been correlated with their octanol-water partition-coefficients at pH 7.4, whereas the extent of binding ~~to P-450 could~~ not be correlated (139). Vermeij (140) has studied the role of lipophilicity on the distribution properties of beta blockers. Tissue storage of propranolol was found to be 15 times higher in brain than plasma, while it was only 3 times higher for metoprolol and practolol. The uptake of beta blockers by red cells and their plasma binding have also been estimated from their lipophilicities (141). Van Zwieten and Timmermans (142, 143) found a correlation between degree of penetration of beta blockers in cerebrospinal fluid and log K for metoprolol, sotalol and atenolol although there was no correlation between their degree of penetration and acute hypotensive effects.

A number of proposals have been made to relate physical properties and molecular structure to cardioselectivity. It has been argued that liposolubility should favor such access of drug to receptor site (144) and that structural features such as the $-OCH_3$ moiety in the side chain, with an amidic or other hydrogen-binding group in the para ring position, are significant (145, 146). Unfortunately, detailed qualitative comparisons and experimental observations argue against any simple relationship. If water solubility were to confer cardioselectivity, then the nonselective sotalol and nadolol would be expected to have this property. A general relationship between the ratio of fractions of protein-bound to free drug and log K in the n-octanol-buffer (pH 7.4) system demonstrated the lack of any obvious relationship to cardioselectivity that could include all drugs examined (147). Cardioselectivity of beta blockers does not appear to be related to any simple physical property which they may possess.

## Nitroimidazoles

The antibiotic azomycin is the simplest biologically active imidazole. It was first isolated in 1953 by the group of Umezawa (148) and identified in 1955 by Nakamura (149) as 2-nitroimidazole. It was found that 2-nitroimidazoles are generally more effective than 5-nitroimidazoles as hypoxic cell radiosensitizers (150, 151). The basic 5-nitroimidazole, nimorazole, is more active on a molar concentration basis than metronidazole (152) while several (2-nitro-1 imidazolyl) alkylamines show correspondingly higher activity than misonidazole (153). These observations have provided the basis for the rational drug design and synthesis of several new series of imidazoles having the potential for improved therapeutic properties as hypoxic cell radiosensitizers compared to metronidazole and misonidazole.

### Structure-Activity Considerations

The most important chemical property in the selection and design of "oxygen-mimicking" radiosensitizers has been electrophilicity. Adams and Cooke (154) suggested that electron affinity was the dominant property that conferred radiosensitizing potential on chemicals. Electron affinity had previously been invoked in a mechanism proposed to explain the radiosensitizing effect of molecular oxygen (155). Chapman et. al. (156) showed that a threshold in electron affinity near that of nitrobenzene existed for the radiosensitization of hypoxic Chinese hamster lung fibroblasts growing *in vitro*. This observation led to a systematic study of the radiosensitizing activity of several substituted nitrobenzenes and resulted in the first experimental confirmation that sensitizing activity correlated strongly with electrophilicity (157).

Lipophilicity of nitroaromatic drugs did not correlate with either toxicity or sensitizing effectiveness in studies with mammalian cells growing *in vitro*(156-158). On the other hand, early studies on the pharmacokinetics and distribution of sensitizers in mice

(159) indicated that lipophilicity could be an important factor. Recent studies by Brown et al. (160) and Brown and Lee (161) on a series of 2-nitroimidazole compounds of similar electron affinity but varying lipophilicity have clearly shown that lipophilicity is an important chemical property for determining drug concentrations in brain and other nervous tissues.

# III. EXPERIMENTAL

## A. MATERIALS

Propranolol(PPL)[1], Acebutolol(ABL)[2], Metoprolol(MPL) and Oxprenolol(OPL)[3], Bupranolol(BPL)[4], Toliprolol(TPL)[5], and Alprenolol(APL)[6], were received and used as their hydrochloride salts. Atenolol(ATL)[7], Nadolol(NDL)[8] and Pindolol(PDL)[9] were received as their free bases. SR-2508, SR-2555, RO-07-2044, Misonidazole, Desmethylmisonidazole, Azomycin riboside, Iodoazomycin riboside[10], Azomycin and RO-07-0741[11] were used as received. L-α-dimiristoylphosphatidylcholine (DMPC 98%), L-α-dipalmitoylphosphatidylcholine (DPPC, 99%) cholesterol (CHOL) and dicetylphosphate (DCP 99%) were used as received[12].

Petroleum ether, chloroform[13], methanol[14] and n-octanol[15] were all reagent grade. Highly pure grades of deuterium oxide ($D_2O$ 99.8% gold label) and praseodymium chloride heptahydrate ($PrCl_3$, 99.99% gold label)[16] were used in the NMR studies. B.D.H. Chemicals[17], De-ionized, distilled water was used to prepare all aqueous solutions.

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[1] Ayerst Laboratories, Montreal, Canada.
[2] May and Baker Ltd., England.
[3] Ciba-Geigy Canada, Ltd.
[4] Sanol Schwartz GmbH.
[5] Boehringer - Ingelheim Canada, Ltd.
[6] Hassle, Sweeden.
[7] ICI, England.
[8] Squibb Canada, Inc.
[9] Sandoz Canada, Ltd.
[10] Dr. L. I. Wiebe Faculty of Pharmacy and Pharmaceutical Sciences University of Alberta, Edmonton, Canada.
[11] Dr. J. D. Chapman Cross Cancer Institute, Edmonton, Alberta Canada.
[12] Sigma Chemical Co., St. Louise, Missouri, USA.
[13] Fisher Scientific Co. NJ
[14] Caledon Lab. Canada
[15] BDH, Toronto, Canada.

## B. METHODS

### UV Analysis of Beta Blockers

Appropriate amounts of beta blockers were dissolved in phosphate buffer (5-43 mg) and diluted to 100 ml at pH 7.4. Further dilutions were made with buffer to obtain concentrations ranging from 0.596 to 430 mcg/ml. The absorbance of the solutions were measured at the $\lambda max$ of each beta blocker and calibration curves were prepared and their linearities described by regression analysis.

### Distribution Studies of Beta Blockers in the n-Octanol-Buffer System

#### Equilibrium Studies of PPL

5 ml of 0.2 mM propranolol in isotonic phosphate buffer, pH 7.4 and 0.5 ml n-octanol, each mutually saturated, were weighed[18] into 25 ml round-bottom flasks and maintained at constant temperature (30° C) in a shaking water-bath[19]. Concentrations of drug in the

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[16] Aldrich Chemical Company, Inc. Milwaukee, WIS.

[17] BDH Chemicals, Toronto, Canada.

[18] Model FP52, Mettler Analytical and Precision Balances, Zurich, Switzerland.

[19] Dubnoff Metabolic Shaking Incubator, Precision Scientific Co., Chicago, IL 60647 USA.

aqueous phase were determined at different time intervals by UV spectrophotometry at 288 nm. The concentration of drug in the oil phase was determined from the mass balance. The distribution of the drug was calculated at various time intervals and the time required to equilibrate the sample was determined.

### Influence of the Oil-Water Phase Volume Ratio

Volumes of n-octanol (0.5-5 ml) and 5 ml of 0.2 mM PPL solution, each mutually-saturated, were weighed into 25 ml round-bottom flasks and equilibrated for 4 hr at 30°C. Concentrations of drug in the aqueous phase were determined by UV spectrophotometry at 288 nm. The concentration of drug in the oil phase was determined from the mass balance and the distribution of the drug was calculated.

### Influence of the Concentrations of PPL, MPL, and TPL

Aqueous phase (5 ml isotonic phosphate buffer, pH 7.4) containing the appropriate concentrations of drug (0.2-20 mM) and n-octanol (0.5 ml) were weighed into 25 ml round-bottom flasks and equilibrated for 4 hr at 37° C. The concentration of drug in the oil phase was determined from the mass balance and the distribution of drug was calculated for the different concentrations of beta blockers.

Influence of the pH of PPL or MPL Solutions

Aqueous phase (5 ml isotonic phosphate buffer, pH 3-9) containing the appropriate concentrations of drug (0.2-0.7 mM) and n-octanol (0.5 ml) were weighed into 25 ml round-bottom flasks and equilibrated for 4 hr at 30° C. Concentrations of drug in the aqueous phase were determined by UV spectrophotometry at λmax. Concentrations of drug in the oil phase were determined from the mass balance and the distribution of the drug was calculated at each solution pH.

Temperature-Dependent Studies

Aqueous phase (5 ml isotonic phosphate buffer, pH 7.4) containing the appropriate concentrations of drug (0.2-1.4 mM) and n-octanol (0.5 ml) were weighed into 25 ml round-bottom flasks and equilibrated for 4 hr at constant temperature ( ±0.5°) in a shaking water-bath[20]. Each phase had been mutually pre-equilibrated with the other at each temperature beforehand. Concentrations of drug in the aqueous phase were determined by UV spectrophotometry[21] at λ max. The concentration of drug in the oil phase was determined from the mass balance. The distribution of the drug was obtained from the average of duplicate determinations at each temperature over the range 10-50° C.

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[20] Dubnoff Metabolic Shaking Incubator, Precision Scientific Co., Chicago, IL 60647 USA

[21] Pye Unicam SP6-550 spectrophotometer, Cambridge, England, CB1 2PX

## **Distribution Studies of Beta Blockers in Liposomes**

### Equilibrium Studies of PPL

DMPC films were formed on the walls of 50 ml round-bottom flasks following rotary evaporation[22] of 5 ml aliquots of a petroleum ether-methanol stock solution (10 mg/ml). The films were dried in a vacuum oven[23] at 30° C overnight. The films were dispersed in 5 ml aliquots of isotonic buffer solution (pH 7.4) at about 40° C, to which 0.2 mM propranolol had been added, by vortex[24] mixing for 10 min. This resulted in the formation of multilamellar liposomes (MLVs). The distribution of the drug was determined at different time intervals of equilibration following centrifugation (143,000 g, 30 min)[25] from UV analysis and mass balance calculations. Determinations were made in duplicate and the results averaged.

### Influence of the Concentrations of PPL, MPL and TPL

These experiments were carried out exactly under the same conditions as above, except that different concentrations of drug (0.2-20 mM) in the aqueous buffer solutions(pH 7.4) were added.

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[22] Lab-Line Instruments Inc. Melrose Park, Illinois, USA.
[23] Model 524, Precision Scientific Co. Chicago, IL 60647 USA.
[24] Deluxe Mixer, Canlab
[25] Model L8-55, Ultracentrifuge, Beckman Instruments, Palo-Alto, CA 94304, USA.

### Influence of Liposome Composition

The experiments were carried out exactly under the same conditions as above, except different liposome compositions, such as DMPC, DPPC, DMPC:CHOL(1:1 mole ratio), DMPC:DCP(7:1 mole ratio) and DMPC:CHOL:DCP(7:2:1 mole ratio) were used.

### Influence of the DMPC:CHOL Ratio

The experiments were carried out exacly under the same conditions as above, except for the liposome composition, such as different ratios of DMPC:CHOL, were used.

### Temperature-Dependent Studies

These experiments were carried out exactly under the same conditions as above, except for the liposome composition and the temperature. In these studies DMPC liposomes were used and partitioning of all the beta blockers were measured at temperatures ranging from 10-41° C.

## UV Analysis of Nitroimidazoles

Ten milligrams of each nitroimidazole was dissolved in methanol and diluted to 100 ml with methanol. Appropriate amounts of stock solution (0.42-1.44 ml) were transferred to 25 ml round bottom flasks, the methanol was evaporated then the nitroimidazoles were dissolved in 2 ml of 0.15 M NaCl.

The absorbance of the solutions were measured[16] at λmax of each nitroimidazole and calibration curves were prepared and their linearities described by regression analysis.

### Distribution Studies of Nitroimidazoles

The experiments were carried out exactly under the same conditions as before, except for the liposome composition, the temperature and incorporation of the drug. In these studies the drug was incorporated in the phospholipid film and the partitioning was measured in DMPC, DPPC, DMPC:CHOL(1:1 mole ratio) and DMPC:CHOL:DCP(7:2:1 mole ratio) liposomes at 30° C.

### Determination of Partition Coefficients

The apparent molal partition coefficients, $K'_m$, were calculated from the distribution results by employing Eq. 24.

$$K'_m = \frac{(C_T - C_w)\, W_1}{C_w\, W_2} \qquad (24)$$

where $C_T$=the total initial concentration of drug (mg/ml) in the aqueous buffer phase before equilibration, $C_w$=final aqueous concentration of drug (mg/ml), $W_1$= weight (g) of aqueous phase, and $W_2$=weight (g) of the lipid phase in the sample.

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[16] Model 25 Beckman Spectrophotometer, Scientific Instruments Division, Irvine CA 92713 USA.

Values of $K'_m$ were converted to $K_m$, using Eq. 25.

$$K_m = K'_m \left( 1 + 10^{pK_a - 7.4} \right) \tag{25}$$

using literature values of the $pK_a$ of each beta blocker.

**Thermodynamics of Partitioning**

The standard change in free energy, $\Delta G^o_{W \rightarrow L}$ due to partitioning is given by

$$\Delta G^0_{W \rightarrow L} = - 2.3\, RT \log K_m \tag{26}$$

The temperature dependence of partitioning was employed to obtain data on the enthalpy of the process based on the relationship (27)

$$\left( \delta H^0_{W \rightarrow L} / \delta T \right)_p = T \left( \delta S^0_{W \rightarrow L} / \delta T \right)_p \tag{27}$$

The assumption is made that $\Delta H^o_{W \rightarrow L}$ and $\Delta S^o_{W \rightarrow L}$ are approximately independent of temperature over the range of interest. Since

$$\log K_m = \frac{\Delta H^0_{W \rightarrow L}}{2.3\, RT} + \frac{\Delta S^0_{W \rightarrow L}}{2.3\, R} \tag{28}$$

a linear plot of log $K_m$ versus 1/T yields $\Delta H^o_{W \rightarrow L}$ from the slope. The change in entropy of partitioning, $\Delta S^o_{W \rightarrow L}$ was obtained from

$$\Delta S^0_{W \rightarrow L} = \frac{\Delta H^0_{W \rightarrow L} - \Delta G^0_{W \rightarrow L}}{T} \qquad (29)$$

The physical meaning of $\Delta H^o_{W \rightarrow L}$ and $\Delta S^o_{W \rightarrow L}$ has been previously defined (page 20 and 21).

## Solution Calorimetry

### Standardization with TRIS

A sample of (TRIS) was dissolved in dilute HCl in a controlled reaction for which the amount of heat evolved is known. According to the recommended calorimeter[17] standardization procedure, 0.5 g of TRIS was dissolved in 100 ml of 0.1 N HCl to evolve 58.738 calories per gram of TRIS at 25° C.

The Dewar flask was tared on a balance and exactly 100 ± 0.05 g of 0.1 N HCl was added. 0.50 ± 0.01 g of TRIS was weighed into the 126C Teflon dish using an analytical balance[18] to an accuracy of 0.0001 g. The rotating cell was assembled and placed in the calorimeter and the motor was started. At equilibrium the recorder[39] was set at 0.10 V(1.000 °C)full scale which was at the bottom of the chart for an exothermic reaction and the thermogram was traced. The thermogram was analyzed to determine the net corrected temperature rise, $\Delta T_c$. The known energy input was calculated by appropriate substitution in Eq. 30.

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[17] PARR 1451 Solution Calorimeter, Moline, Illinois 61265 USA.

[18] Model AE163, Mettler Analytical and Precision Balances, Zurich, Switzerland.

$$Q_E = m\left[\, 58.738 + 0.3433\,(25 - T_{0.63R})\,\right] \tag{30}$$

where $Q_E$ =the enegy input in calories, m=the weight of TRIS in grams, and T=the temperature at the 0.63R point on the thermogram. The term 0.3433 $(25 - T_{0.63\,R})$ adjusts the heat of reaction to any temperature above or below the 25° C reference temperature. The energy equivalent of the calorimeter and its contents were calculated by substitution into Eq. 31.

$$e = \frac{Q_E}{\Delta T_c} \tag{31}$$

where 'e' is expressed in calories per °C. The energy equivalent of the empty calorimeter was determined by subtracting the heat capacity of the 100 g of 0.1 N HCl from 'e' as follows:

$$e' = e - (100.00)\,(0.99894) \tag{32}$$

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[39] Fisher Recordall Series 5000, Fisher Scientific Co. PA 15719 USA.

where e'=the energy equivalent of the empty calorimeter in calories per °C. 100.00= the grams of 0.1 N HCl and 0.99894= the specific heat of 0.1 N HCl at 25° C.

### Determination of the Enthalpy of Solution ($\Delta H^\circ_w$) of Propranolol in Phosphate Buffer at pH 5.0

Phosphate buffer saturated with n-octanol (100.00±0.05 g) was added to the tared Dewar flask, then 0.10 g of propranolol(free base) was weighed into the 126C Teflon dish on an analytical balance. It was necessary to conduct studies at pH 5.0 to avoid the solubility problem of propranolol free base in phosphate buffer.

The rotating cell was assembled, and placed in the calorimeter, the motor started, and at equilibrium the recorder was set at 0.01 V full scale (0.100 °C). The base line was set at the bottom of the chart for an exothermic reaction and the thermogram was traced. Triplicate samples were recorded. Thermograms were examined to determine the net corrected temperature rise, $\Delta T_c$. The enthalpy change, $\Delta H$, of propranolol was calculated from Eq. 33.

$$\Delta H = \frac{-Q}{m} \qquad (33)$$

where $\Delta H$=the enthalpy change, Q=the energy evolved, and m=the weight of propranolol. The energy evolved 'Q' was calculated from Eq. 34.

$$Q = (\Delta T_c)(c) \qquad (34)$$

where $\Delta T_c$ = the corrected temperature rise and e=the energy equivalent obtained from the calibration of the instrument.

### Determination of the Enthalpy of Solution ($\Delta H_O^\circ$) of Propranolol in n-Octanol

n-Octanol (100.00±0.05 g) saturated with phosphate buffer (pH 5.0) was weighed into the tared Dewar flask and 0.10 g of propranolol(free base) was added to the 126C Teflon dish. The rotating cell was assembled as before and placed in the calorimeter and the motor was started. When the calorimeter reached equilibrium the recorder was set at 0.01 V full scale (0.100 °C), then the base line was set at the top of the chart for an endothermic reaction and the thermogram traced. Triplicate samples of propranolol 0.10 g were recorded. Thermograms were examined to determine the net corrected temperature change, $\Delta T_c$ then $\Delta H_O^\circ$, was calculated using Eqs. 30-33.

### Determination of the Enthalpy ($\Delta H_{W\rightarrow O}^\circ$) of Transfer of Propranolol from Aqueous Solution to n-Octanol Phase

The enthalpy of transfer, $\Delta H_{W\rightarrow O}^\circ$, was calculated using an Equation 17.

## Uptake Studies of Propranolol

### In Erythrocytes

Fresh human blood to which ethylenediamine-tetraacetic acid (EDTA) had been added as an anticoagulent was obtained from the University of Alberta Hospital for each series of experiments. Prior to each experiment, the blood was washed three times in pH 7.4 isotonic Tris buffer, then diluted to yield a test sample containing 5-7 percent hematocrit. The test sample (2 ml) was diluted with 1 ml PPL solution, divided into two equal portions then transferred to centrifuge tubes (1.5 ml Eppendorf), vortex-mixed, incubated at the desired temperature for 15 min, then centrifuged[16] at 1500 rpm for 1 min. The supernatant was carefully removed and its absorbance measured at 288 nm against buffer solution as reference. Absorbance values were corrected for the absorbance of a blank treated similarly. Experiments with several blanks gave the same absorbance value verifying that the amount of absorbing material retained in the supernatant was reasonably constant on all occasions. Concentrations of PPL were obtained from a calibration curve and the uptake determined. The temperature dependent studies were conducted at 25°, 30°, and 37° C and concentration-dependent studies covered the range 0.1 to

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[16] Fisher Microcentrifuge, Model 235B, Fisher Scientific, Co. Pittsburgh, PA, USA.

2.3 mM PPL. The results were averaged from quadruplicate experiments.

### In Erythrocyte Ghosts

Erythocyte ghosts were prepared from fresh human blood as follows: Whole blood was centrifuged at 2500 rpm for 5 min, and washed twice with saline. The pooled, packed cells were diluted 14 times with ice-cold Tris/EDTA solution, mixed for 10 min, then centrifuged (36,000 g, 37°C, 30 min). Subsequently, the supernatant was removed and the pellet resuspended in Tris/EDTA and centrifuged again (36,000 g, 37°C, 15 min). Following this treatment, the pellet was then washed twice in 10 mM Tris buffer and centrifuged as before. Finally, the ghost cells were diluted back to the original volume and this stock suspension was used for the uptake studies. The test sample (4 ml of stock suspension) was diluted with 2 ml of isotonic, buffered PPL solution, vortex-mixed, incubated for 30 min, then centrifuged (56,000 g, 37°C, 10 min). The uptake of PPL by the ghost cells was determined by difference from the residual concentrations of the supernatant. The results of triplicate experiments were averaged.

### In Liposomes

Liposomes were prepared by a modified Bangham method (63). A thin film of the lipid components was formed on the wall of a round-bottom flask then dispersed in 5 ml of an aqueous, buffered, solution of PPL at approximately 40° C, vortex-mixed for 10 min, then 3.5 ml were transferred to a centrifuge tube. The liposomes were equilibrated at the desired temperature for at least 24 hr, centrifuged (143,000 g, 37°C, 30 min) and the concentration of drug in the supernatant determined spectrophotometrically. Under these conditions, the residual phospholipid in the supernatant is negligible (31, 167).

## Partition Coefficient Determinations

Apparent partition coefficients ($K'_m$) of propranolol were determined in erythrocytes, erythrocyte ghosts, and liposomes from uptake studies. $K'_m$ were expressed as mole(Kg dry membrane)$^{-1}$/mole $l^{-1}$. The dry weight of erythrocytes was determined as follows: To 0.5 ml of the packed erythrocytes 1.5 ml distilled water was added, mixed for 3 minutes to hemolyse the erythrocytes and 1.5 ml of Tris buffer twice the concentration was added and mixed to seal the hemolysed erythrocytes. Centrifuged at 10,000 rpm for 10 minutes and the supernatant was removed. The volume of erythrocyte suspension was adjusted to 25 ml with distilled water. 10 ml of the diluted suspension was dried in oven at 100° C for 24 hours and the dry weight was determined. The dry weights of erythrocytes and erythrocyte ghosts were found to be 2.9% and 0.78% respectively. In temperature-dependent studies individual samples were used for erythrocytes or ghosts at each temperature, whereas in liposome systems same samples were repeatedly equilibrated at different temperatures to determine $K'_m$ values.

### Phospholipid Analysis

The phospholipid content of erythrocytes and liposome was determined by phosphate analysis as described by Fiske and Subbarow (197). subsequently, the phospholipid content of erythrocytes was expressed in terms of equivalent DMPC. Thus, an average of 15.93 μmoles phosphate/ml of erythrocytes calculated for an equivalent of 100% hematocrit was found (4 experiments) which is equivalent to 24.69 μmoles phospholipid/ml of erythrocytes. Erythrocyte ghosts were assumed to contain an equivalent amount of phospholipid as for erythrocytes. In this way, uptake of propranolol could be compared among erythrocytes, ghosts and liposomes on a normalized basis in terms of phospholipid.

## Antihemolysis Studies of Erythrocytes Employing Propranolol

The stabilization of the erythrocyte membrane against hemolysis by PPL addition was measured at 37° C at various concentrations of drug according to the procedure reported by Florence and Rahman (162). Erythrocyte suspensions for hemolysis experiments were prepared by centrifuging citrated blood at 1500 g for 10 min. The plasma was carefully removed and 1 ml of the erythrocyte layer was mixed with 154 mM NaCl in 10 mM phosphate buffer at pH 7, to give a total volume of 12.5 ml. This served as stock suspension. Hemolysis experiments were carried out as follows:

0.1 ml of the stock erythrocyte suspension was added to 1 ml of 130 mM NaCl in 20 mM sodium phosphate buffer, pH 7.4, with 7 ml of PPL solution (0.01-10 mM)in water. In control experiments water was added instead of PPL solution. The mixture was kept at room temperature for 5 min and then centrifuged for 45s at 1500 g. The hemoglobin content of the clear supernatant was measured by recording the absorbance at 543 nm using a spectrophotometer. All experiments were conducted at least in triplicate and the results averaged.

## Proton NMR Studies of the Interactions of Beta Blockers with Unilamellar Liposomes

### Preparation of Beta Blockers in $D_2O$

Six beta blockers were investigated in this study, namely PPL, OPL, APL, TPL, MPL and ATL. Each dissolved in $D_2O$ to make 10 ml of solution. Concentrations were prepared over the range 10-40 mM.

### Preparation of $PrCl_3$ in $D_2O$

75 mg of $PrCl_3$ heptahydrate were dissolved in 2 ml $D_2O$ in a 25 ml round-bottom flask. The solution was freeze dried[11] overnight. The resulting film was dissolved in $D_2O$, then transferred to a 10 ml volumetric

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[11] Virtis Research Equipment, Gardiner, New York, USA.

flask to produce 20 mM $PrCl_3$ stock solution.

## Preparation of Liposomes

Films containing 100 mg of DMPC were prepared in round-bottom flasks as before and dried in a vacuum oven at 30° C overnight. The resulting dried films were dispersed in 5 ml $D_2O$ by vortex mixing for 5 min. The reulting MLVs were probe sonicated[18] for 30 min to obtain a clear solution of unilamellar liposomes. The unilamellar liposomes were equilibrated at 30° C in a water-bath until required for use.

## Preparation of Liposomes Containing Beta Blockers

0.5 ml of a unilamellar liposome stock solution were transferred to a 3 ml vial. 0.1 ml of 20 mM $PrCl_3$ solution was added to each vial and then equilibrated at 30° C for 30 min. Solutions of beta blockers were added in various amounts then diluted to 1 ml with $D_2O$. The spectra of these samples were recorded within 15 minutes of preparing the samples at 30° C.

## Determination of NMR Spectra

The proton NMR spectra were recorded with a 300 MHz spectrophotometer[19]. All spectra were recorded at 30° C with a reproducibility of 3 Hz. Chemical shift measurements were carried out using the HDO peak as internal reference which was set at 4.63 ppm.

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[18] W-375, Heat Systems, Ultrasonics, Inc. Plainview, NY 11803 USA.

[19] Bruker AM - 300 MHz spectrophotometer

## Statistical Analysis

1. Relative standard deviation (%) was calculated as shown below:

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

The relative standard deviations (%) were calculated for all the experimental data.

2. Statistics based on the two sided t-test were used to evaluate the significance of the coefficient of determination obtained in the regressions. Levels of $p<0.05$ were considered to be significant.

## IV. RESULTS

### A. ANALYSES OF BETA BLOCKERS

The wavelengths of maximum absorbance, $\lambda$max, of beta blockers in phosphate buffer (pH 7.4), their chemical structures, molecular weights and $pK_a$'s are shown in Table 1. Linear calibration curves which obeyed Beers law over the concentration range of 10-430 mcg/ml were obtained for the beta blockers as shown in Figures 1-4. Regression analysis of the experimental points yielded slopes ranging from 0.003 to 0.027, intercepts from 0.002 to 0.075 and correlation coefficients, r, from 0.999 - 1.000.

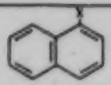
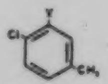
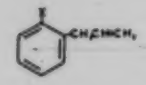
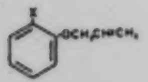
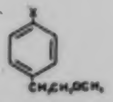
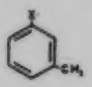
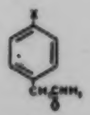
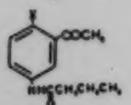
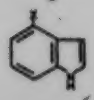
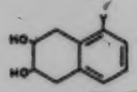
### B. PARTITIONING OF BETA BLOCKERS IN THE n-OCTANOL-BUFFER SYSTEM

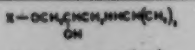
**Temperature Dependence**

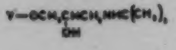
Log K' of beta blockers as a function of temperature are given in Table 2. Van't Hoff plots of the corresponding log K values as shown in Figure 5, indicate a uniform increase in log K with temperature for all of the beta blockers except at low temperatures. Depending on the chemical nature of the drug, PPL, BPL, APL and TPL had lower K' values at 10° than expected while values for ABL, PDL and MPL could not be detected at 10° or for MPL at 20°. Also additional experiments were carri~~ed out~~, varying the phase volume ratios of n-octanol and water and equilibration times

Table 1

Chemical Structures and Physical Properties of Beta Blockers.

| Beta Blocker | Structure | Molecular Weight | $\lambda_{max}$ | $pK_a$ |
|---|---|---|---|---|
| Propranolol (PPL) |  | 259.3 | 288 | 9.45 |
| Bupranolol (BPL) |  | 271.8 | 274 | 9.6 |
| Alprenolol (APL) |  | 249.3 | 270 | 9.7 |
| Oxprenolol (OPL) |  | 265.3 | 272 | 9.5 |
| Metoprolol (MPL) |  | 267.4 | 277 | 9.7 |
| Toliprolol (TPL) |  | 223.3 | 270 | 9.6 |
| Atenolol (ATL) |  | 266.3 | 273 | 9.55 |
| Acebutolol (ABL) |  | 336.4 | 320 | 9.67 |
| Pindolol (PDL) |  | 248.3 | 263 | 8.8 |
| Nadolol (NDL) |  | 308.9 | 276 | 9.67 |


$X{-}OCH_2\underset{OH}{CH}CH_2NHCH(CH_3)_2$


$Y{-}OCH_2\underset{OH}{CH}CH_2NHC(CH_3)_3$

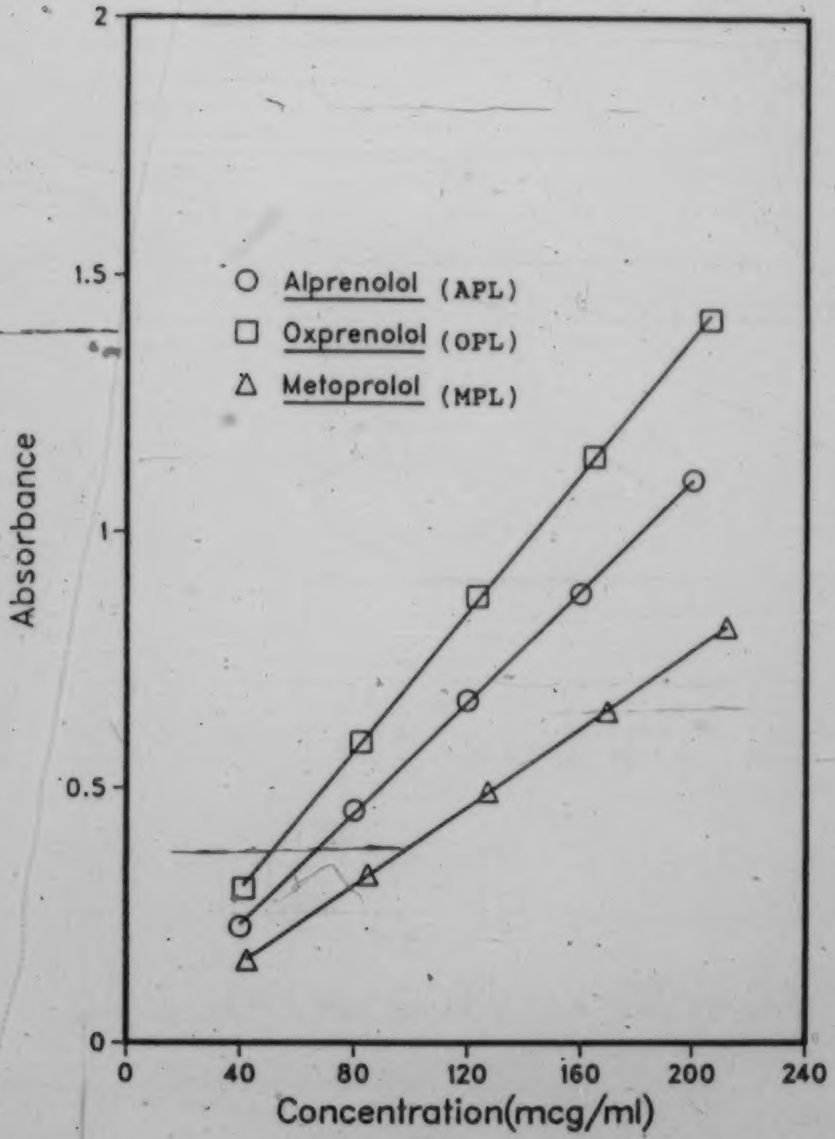


Figure 1. Beers plot of APL, OPL and MPL in phosphate buffer at pH 7.4.

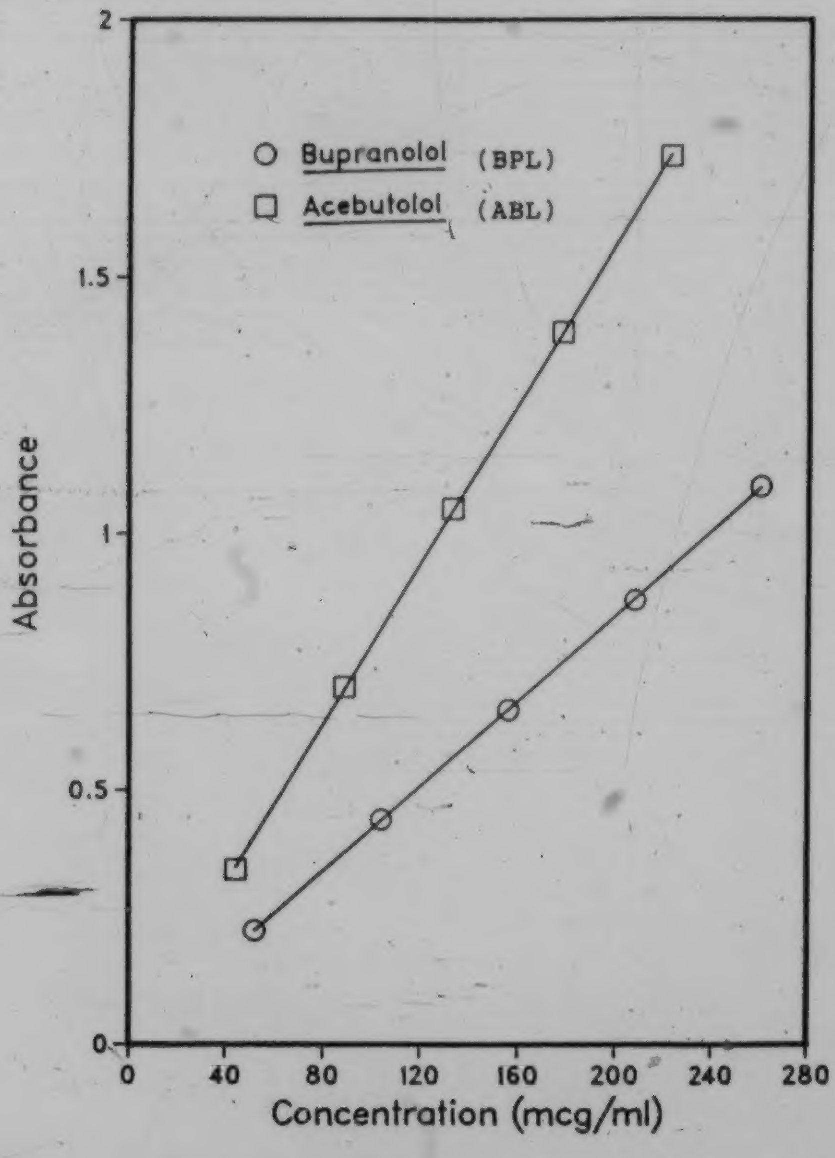


Figure 2. Beers plot of BPL and ABL in phosphate buffer at pH 7.4.

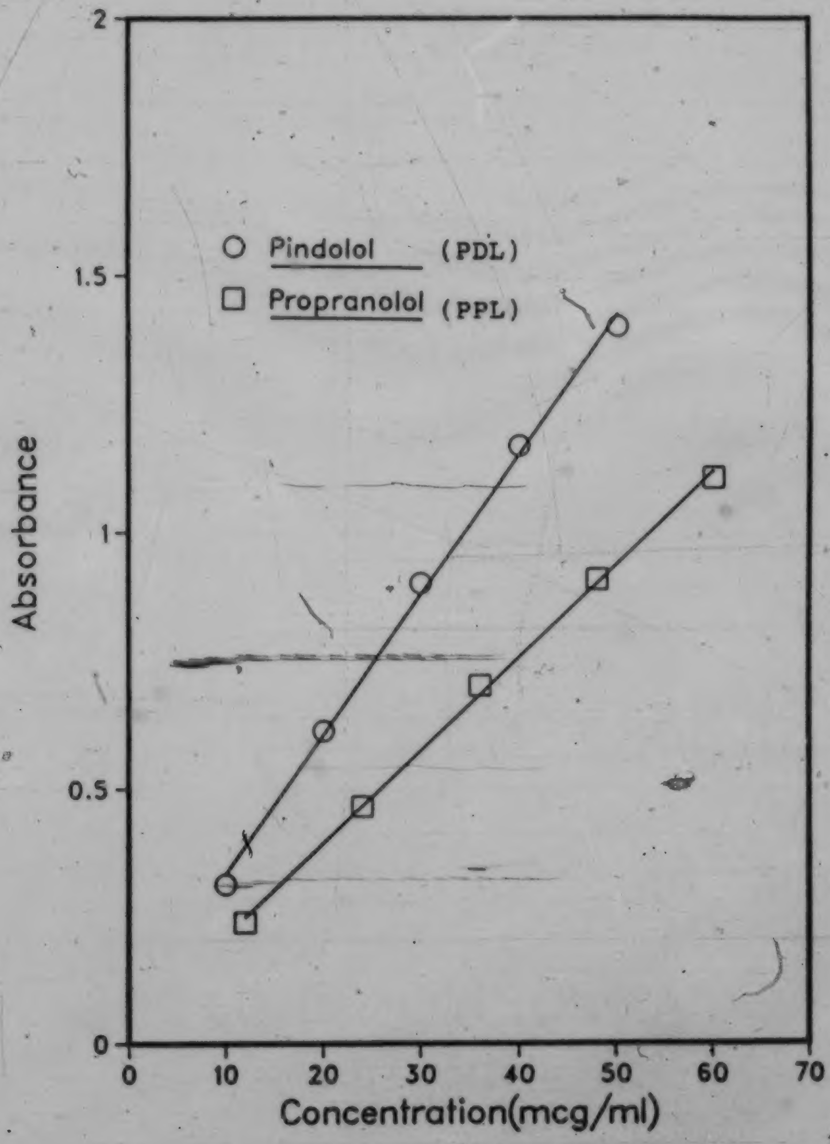


Figure 3. Beers plot of PDL and PPL in phosphate buffer at pH 7.4.

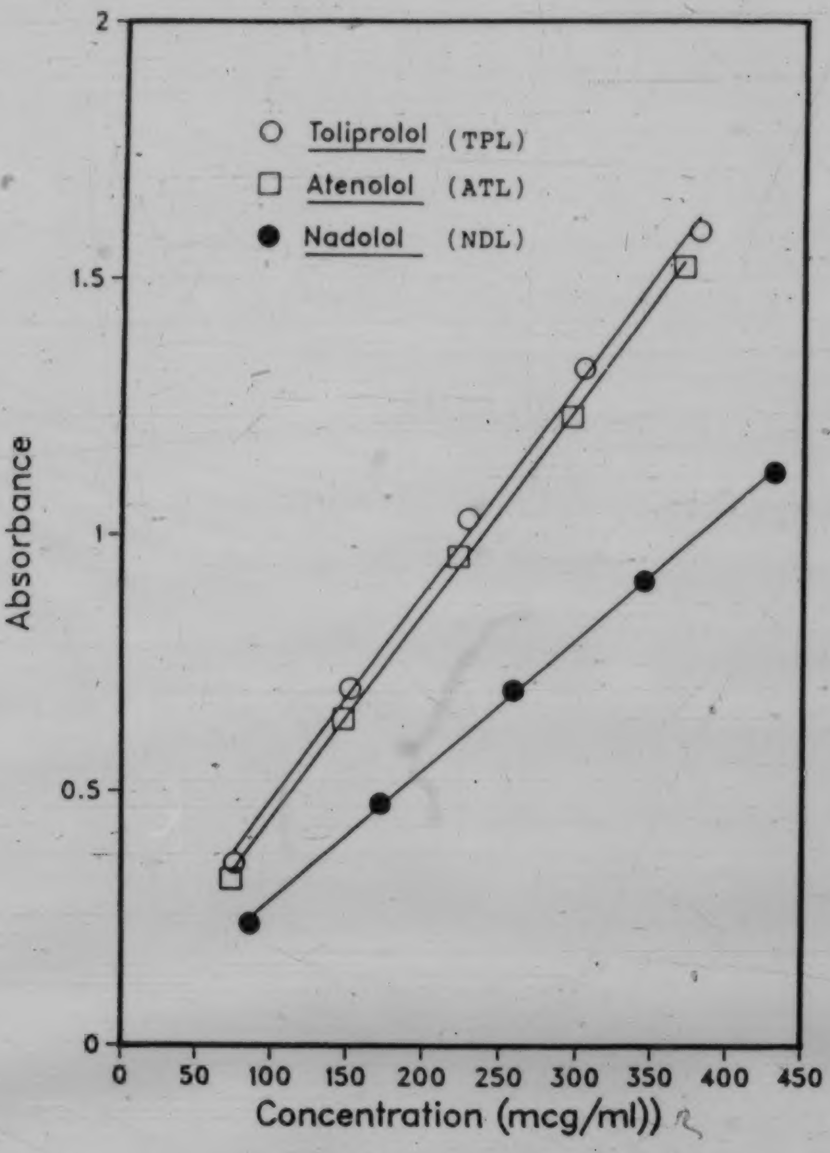


Figure 4. Beers plot of TPL, ATL and NDL in phosphate buffer at pH 7.4.

Table 2

Apparent Partition Coefficients (log K') of Beta Blockers in the n-Octanol-Phosphate Buffer System at pH 7.4[a]

| | Temperature °C | | | | |
|---|---|---|---|---|---|
| Beta Blocker | 10 | 20 | 30 | 40 | 50 |
| Propranolol | 0.65 | 1.12 | 1.30 | 1.53 | 1.66 |
| Bupranolol | 0.09 | 0.81 | 1.08 | 1.23 | 1.46 |
| Alprenolol | 0.53 | 0.93 | 1.16 | 1.34 | 1.53 |
| Oxprenolol | 0.18 | 0.26 | 0.37 | 0.75 | 0.94 |
| Toliprolol | -0.42 | 0.12 | 0.40 | 0.57 | 0.79 |
| Acebutolol | - | -0.74 | 0.15 | 0.61 | 0.71 |
| Metoprolol | - | - | 0.00 | 0.25 | 0.46 |
| Pindolol | - | -0.36 | 0.09 | 0.08 | 0.21 |
| Atenolol | -0.46 | -0.48 | -0.06 | -0.11 | -0.02 |
| Nadolol | - | - | - | - | -1.22 |

[a] The maximum R. S. D. was ±11%. In most cases the R. S. D. was < ±5.4%.

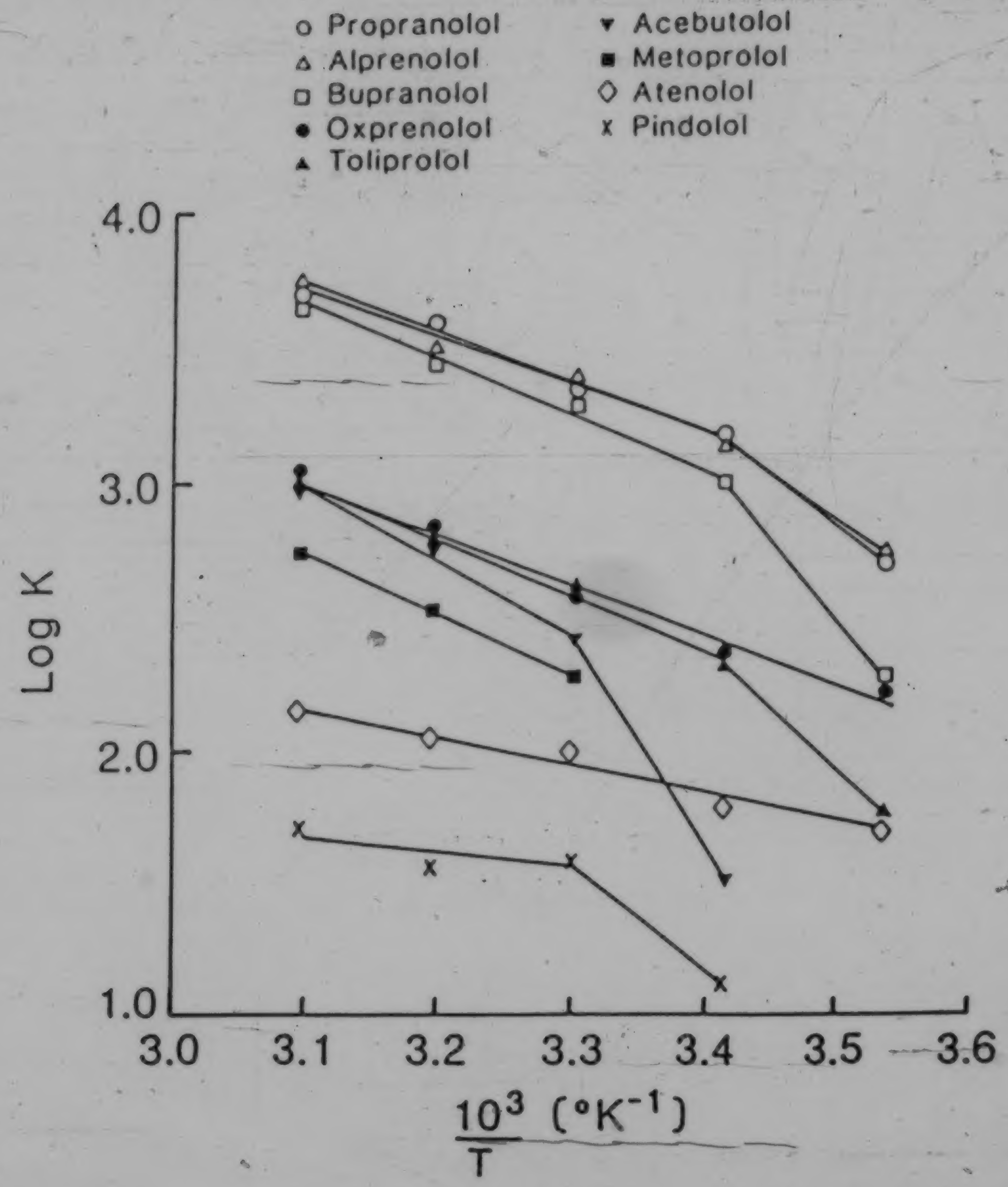


Figure 5. Van't Hoff plots of the partition coefficients of beta blockers in the n-octanol-phosphate buffer system at pH 7.4. (The maximum R. S. D. was ±2.4%. The range of duplicate experimental values does not extend beyond the symbol dimensions in all cases)

to determine ~~that~~ whether under these conditions a K' value could be detected. But this was not the case. On the other hand, the range of standard deviations of K's of ATL and OPL (0.0071-0.0141) were comparable to those of PPL, BPL, APL and TPL (0.0071-.0212) at 10° C and ABL, PDL (0.00-0.0283) at 20° C which suggests the validity of the mean values as differentiated as shown in Figure 5. Nadolol did not yield a measurable K' over the temperature range of 10°-40° but its log K' at 50° was found to be -1.22. The exceptions to this type of behavior were OPL and ATL which yielded linear plots over the entire temperature range. The relative lipophilicities of the beta blockers fall into approximately three groups: PPL, BPL, APL (I); OPL, TPL, ABL, MPL (II); and ATL, PDL (III).

### Concentration Dependence

The effect of beta blocker concentration on partitioning at pH 7.4 and 37° C is summarized in Figure 6. Only a slight dependency of log K on increasing concentrations of PPL, MPL or TPL is observed and in marked contrast to that observed for chlorpromazine in the n-octanol-phosphate buffer system at 37° (118).

### pH Dependence

Figure 7 shows the pH-absorbance profiles of PPL and MPL at 30° C. Their molar absorptivities were found to be independent of pH. At low pH, the absorbance is high because

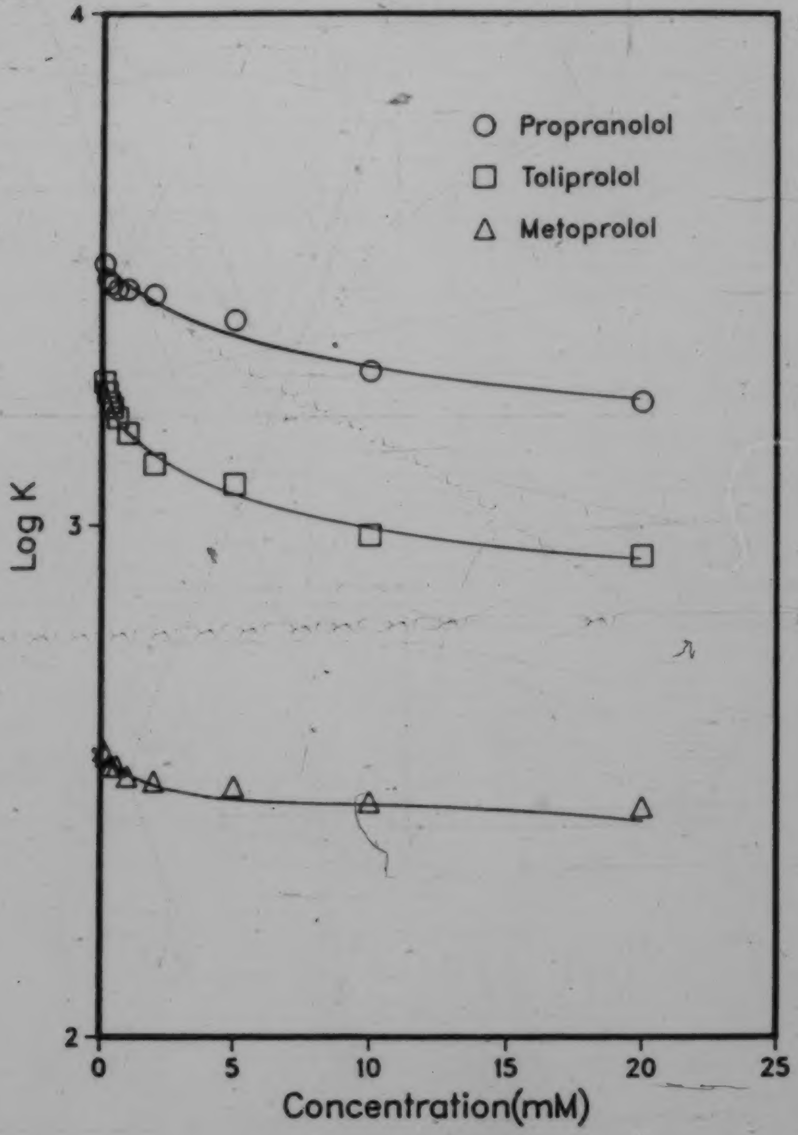


Figure 6. Concentration dependence of partition coefficients of PPL, TPL and MPL in the n-octanol-buffer system at pH 7.4 and 37° C. (The maximum R. S. D. was ±1.7%. The range of duplicate experimental values does not extend beyond the symbol dimensions in all cases)

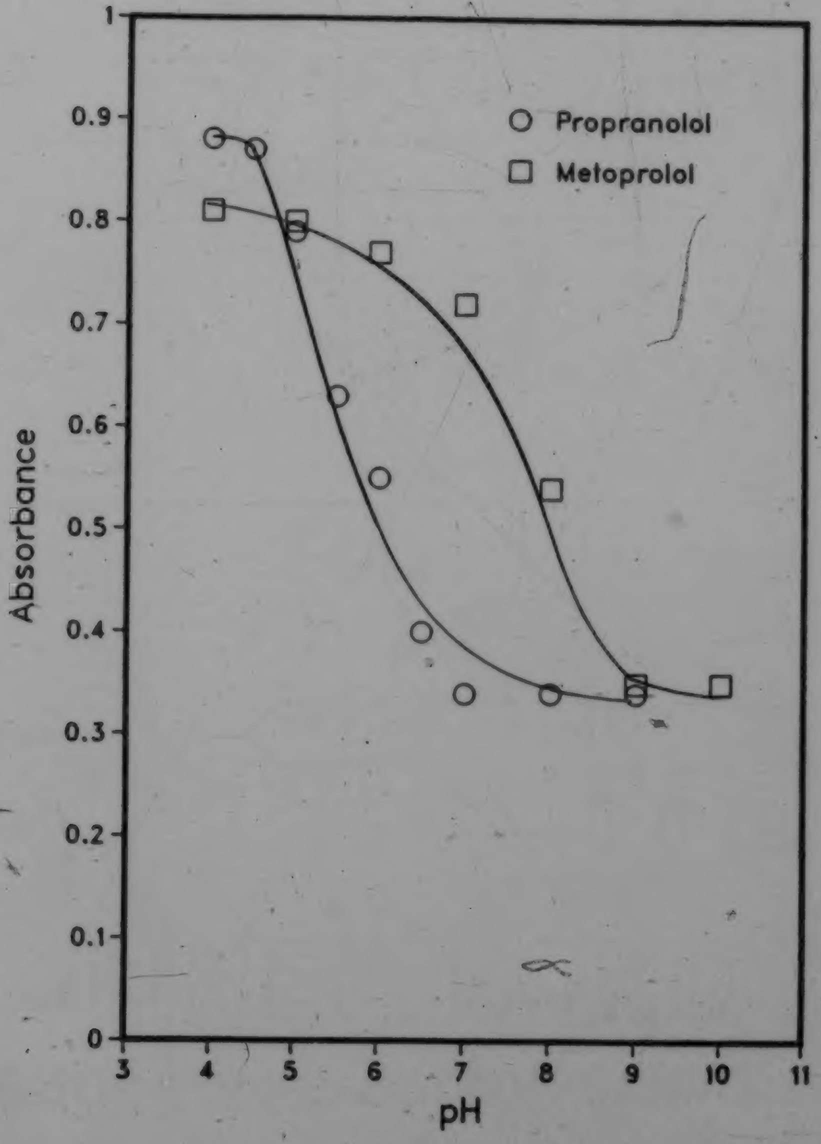


Figure 7. pH dependence of the absorbance of PPL and MPL in the equilibrated aqueous phase of the n-octanol-aqueous partitioning system at 30° C. (The maximum R. S. D. was ±1.05%. The range of duplicate experimental values does not extend beyond the symbol dimensions in all cases)

the drugs are essentially completely ionized and reside in the aqueous phase. However, as the pH was increased the absorbance decreased indicating extraction of unionized drug by n-octanol. A similar pattern of behavior of PPL has previously been reported in the literature (196).

## C. PARTITIONING OF BETA BLOCKERS IN LIPOSOMES

### Temperature Dependence

Log $K'_m$ values of beta blockers as a function of temperature are given in Table 3. Van't Hoff plots of the corresponding log $K_m$ values are shown in Figure 8. It can be seen here that the beta blockers partitioned in liposomes even at low temperatures (except NDL) in contrast to the n-octanol-buffer system. A discontinuity in the curves between low and high temperature ranges in the vicinity of the Tc of DMPC dispersions (23° C) is clearly evident (59). Also, the slopes of the curves decrease with increase in lipophilicity <Tc and >Tc although their inclinations are generally opposite. The obvious exception to this pattern of behavior is NDL which displays an opposite trend >Tc compared to the other beta blockers. However, its partitioning in liposomes <Tc was not detectable. Partitioning studies do not represent a sensitive means of detecting the Tc of a phospholipid but the results in Figure 8 suggest the following: PPL, APL, BPL, TPL, OPL do not change the Tc of DMPC; but MPL, ATL, ABL, PDL cause it to

## Table 3

Log K$'$ Values of Beta Blockers in DMPC Liposomes at pH 7.4[a].

| | Temperature °C | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Beta Blocker | 10 | 15 | 20 | 23 | 27 | 30 | 33 | 37 | 41 |
| Propranolol | 2.15 | 2.09 | 2.08 | 2.28 | 2.66 | 2.68 | 2.70 | 2.62 | 2.62 |
| Bupranolol | 2.04 | 2.03 | 1.99 | 2.20 | 2.47 | 2.51 | 2.51 | 2.51 | 2.55 |
| Alprenolol | 1.84 | 1.93 | 1.87 | 2.01 | 2.22 | 2.25 | 2.26 | 2.23 | 2.26 |
| Toliprolol | 1.19 | 1.10 | 0.88 | 1.23 | 1.46 | 1.51 | 1.47 | 1.54 | 1.54 |
| Pindolol | 1.09 | 1.09 | 1.03 | 1.21 | 1.31 | 1.30 | 1.40 | 1.42 | 1.42 |
| Oxprenolol | 1.07 | 1.09 | 0.88 | 1.23 | 1.48 | 1.51 | 1.54 | 1.54 | 1.57 |
| Metoprolol | 0.90 | 0.88 | 0.72 | 1.00 | 1.12 | 1.22 | 1.21 | 1.23 | 1.33 |
| Atenolol | 0.80 | 0.13 | - | 0.97 | 0.94 | 1.03 | 1.09 | 1.09 | 0.94 |
| Acebutolol | 0.21 | -0.35 | - | 0.44 | 0.61 | 0.71 | 0.69 | 0.65 | 0.78 |
| Nadolol | - | - | - | 0.82 | 1.07 | 0.97 | 0.72 | 0.80 | 0.41 |

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[a] The maximum R. S. D. was ±10%. In most cases the R. S. D. was < ±4.6%.

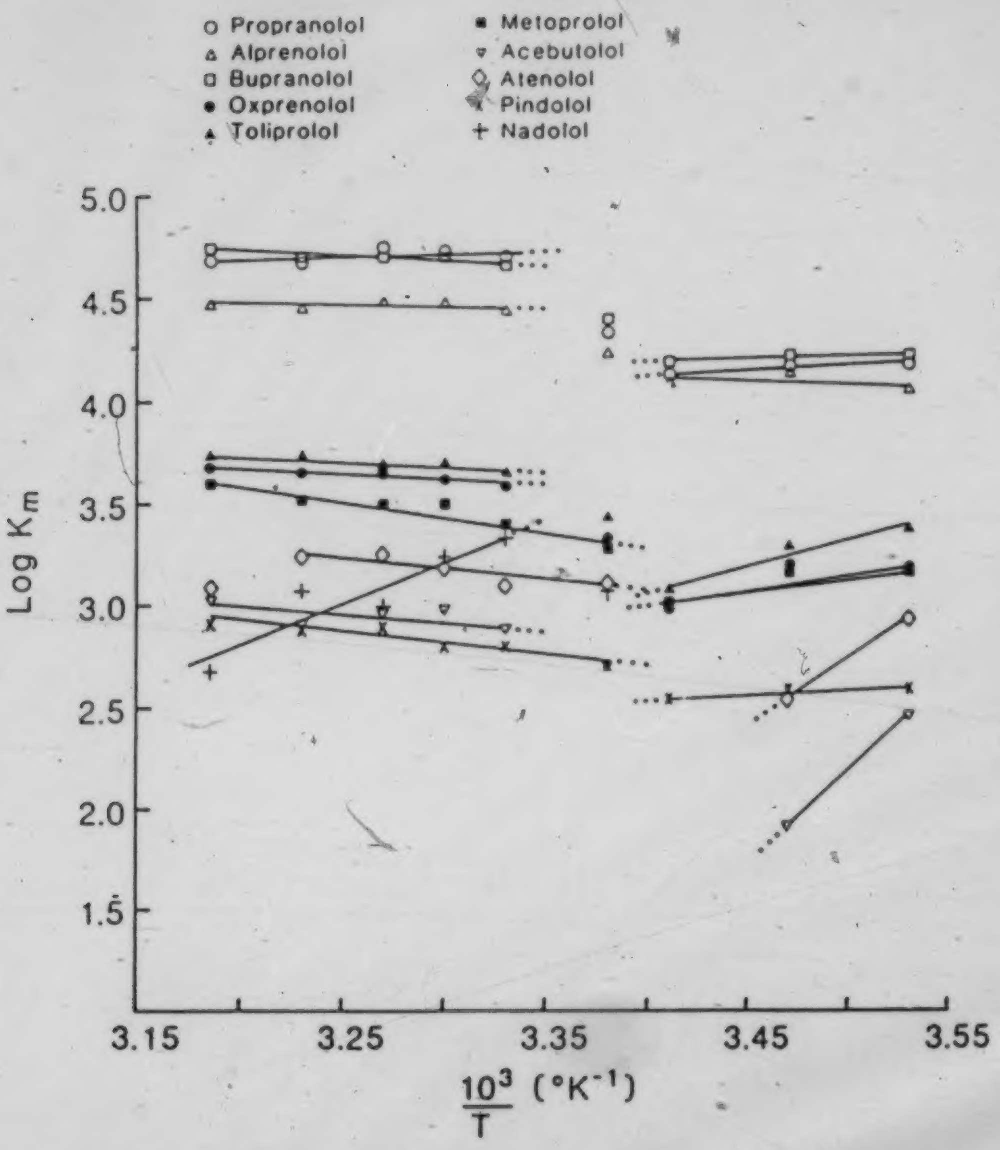


Figure 8. Van't Hoff plots of the partition coefficients of beta blockers in the liposome-phosphate buffer system at pH 7.4. (The maximum R. S. D. was ±1.6%. The range of duplicate experimental values does not extend beyond the symbol dimensions in all cases)

decrease. Also, the greater the decrease in the Tc caused by the solute (e.g. ATL, ABL), the more unfavorable the environment for partitioning in the region of the Tc. Hence, a $K'_m$ could not be measured for ATL and ABL at 20° C.

## Concentration Dependence

The dependencies of log $K'_m$ on the concentrations of PPL, MPL and TPL in DMPC liposomes are shown in Table 4 and the corresponding plots of log $K_m$ are shown in Figure 9. The observed tendency towards decreasing values of log $K_m$, is in marked contrast to that observed for chlorpromazine in the DMPC liposome system at 37° C (118). Alcohols (163) and phenols (164) have been previously shown to behave in a manner similar to the beta blockers.

## Effect of DMPC Concentration

The dependencies of log $K'_m$ and log $K_m$ on increasing DMPC concentrations are shown in Table 5. The partitioning could not be detected at 0.01 - 0.05 mg/ml of DMPC. There was a slight decrease in partitioning with increasing DMPC concentration up to about 1 mg/ml but then remained constant.

## Effect of DMPC:CHOL Ratio

$K_m$ values of PPL determined as a function of DMPC:CHOL ratio are given in Table 6. At high ratios or low concentrations of CHOL it can be seen that the partitioning

Table 4

Effect of Beta Blocker Concentration on Partitioning in DMPC Liposomes at 37°C and pH 7.4.

| Concentration | $^a$Log K | | |
|---|---|---|---|
| (mM) | Propranolol | Toliprolol | Metoprolol |
| 0.1 | 2.87±0.03 | 1.58±0.03 | 1.30±0.03 |
| 0.2 | 2.69±0.01 | 1.54±0.02 | 1.29±0.01 |
| 0.3 | 2.61±0.01 | 1.52±0.02 | 1.29±0.03 |
| 0.4 | 2.58±0.02 | 1.51±0.04 | 1.27±0.04 |
| 0.6 | 2.47±0.04 | 1.50±0.04 | 1.24±0.01 |
| 1.0 | 2.43±0.03 | 1.48±0.03 | 1.06±0.03 |
| 2.0 | 2.38±0.03 | 1.46±0.01 | 0.96±0.06 |
| 5.0 | 2.05±0.04 | 1.44±0.01 | 0.93±0.03 |
| 10.0 | 1.85±0.02 | 1.35±0.03 | 0.90±0.03 |
| 20.0 | 1.68±0.04 | 1.23±0.01 | 0.76±0.04 |

[a] Mean±S.D.

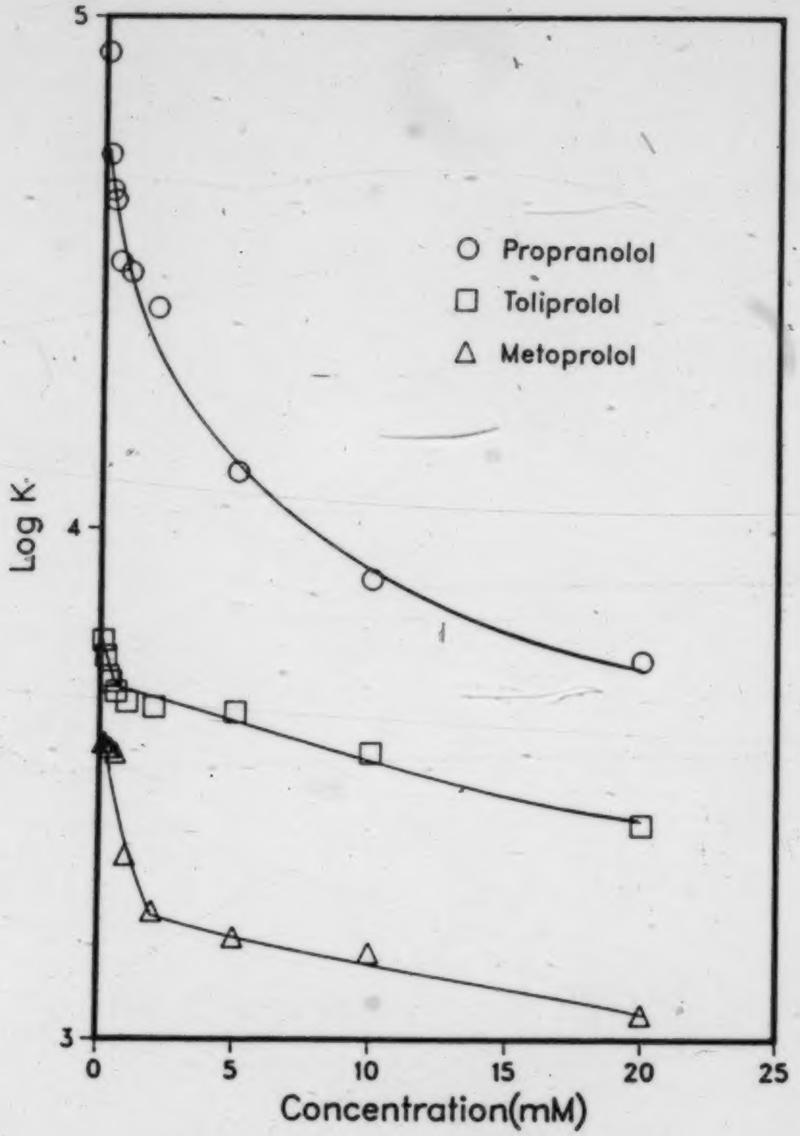


Figure 9. The concentration dependence of log K of PPL, TPL and MPL in DMPC liposomes at pH 7.4 and 37° C. (The maximum R. S. D. was ±1.9%. The range of duplicate experimental values does not extend beyond the symbol dimensions in all cases)

Table 5

Effect of DMPC Concentration on the Partitioning of Propranolol at 37° C and pH 7.4.

| DMPC (mg/ml) | [a]log $K'_m$ | [a]log $K_m$ |
|---|---|---|
| 0.01 | - | - |
| 0.02 | - | - |
| 0.05 | - | - |
| 0.10 | 2.80±0.04 | 4.85±0.05 |
| 0.20 | 2.80±0.06 | 4.85±0.07 |
| 0.50 | 2.72±0.03 | 4.78±0.03 |
| 1.00 | 2.64±0.04 | 4.69±0.04 |
| 2.00 | 2.60±0.03 | 4.66±0.03 |
| 5.00 | 2.61±0.01 | 4.67±0.01 |
| 10.00 | 2.62±0.03 | 4.67±0.04 |

[a] Mean±S.D.

## Table 6

Effect of CHOL Content in DMPC Liposomes on Partitioning of Propranolol at 37° C and pH 7.4.

| DMPC:CHOL mole ratio | $^{a}K_m$ (x$10^{4}$) |
|---|---|
| 1:0 | 4.68±0.10 |
| 8:1 | 6.31±0.12 |
| 4:1 | 2.51±0.06 |
| 2:1 | 1.23±0.06 |
| 1:1 | 0.37±0.07 |

[a] Mean±S.D.

of PPL was actually increased compared to DMPC alone but as the concentration of CHOL in the liposomes was increased the partitioning of PPL decreased fairly sharply.

### Effect of Liposome Composition

The log $K'$ values of beta blockers in the n-octanol-buffer system and log $K'_m$ values in liposomes of various phospholipid compositions at 37° C and pH 7.4 ~~are~~ compared in Table 7. Log $K'_m$ and log $K_m$ values of beta blockers in all liposome compositions were higher than in the n-octanol-buffer system. Partitioning of NDL could not be detected in the n-octanol-buffer system and in DMPC:CHOL (1:1 mole ratio) liposomes. For the others, partitioning was comparatively low in DMPC:CHOL (1:1 mole ratio) liposomes but high in DMPC:DCP (7:1 mole ratio) and DMPC:CHOL:DCP (7:2:1 mole ratio) liposomes.

## D. FUNCTIONAL GROUP CONTRIBUTIONS

Hydrophobic substituent constants have been derived from Eq. 11, in order to compare the relative affinities of the various functional groups for the n-octanol-buffer system and DMPC liposomes. These values, shown in Table 8 were obtained from the values of K or $K_m$ of the various beta blockers (x) and from the parent compound, pre-prenalterol (H). In every case $\pi_x$ (liposome) is greater than $\pi_x$ (n-octanol) and on an average, it was 55% greater.

Table 7

Log K' (n-Octanol-Buffer) of Beta Blockers and log K (Liposomes) at 37° C and pH 7.4.[a]

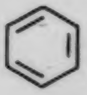
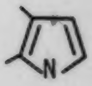
| Beta Blocker | n-Octanol-Buffer System | Liposome Composition | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| Nadolol | - | 0.80 | 0.64 | - | 1.00 | 0.87 |
| Pindolol | 0.08 | 1.42 | 1.30 | 0.49 | 1.17 | 1.58 |
| Atenolol | 0.11 | 1.09 | 0.32 | 0.56 | 0.37 | 1.43 |
| Metoprolol | 0.20 | 1.23 | 1.28 | 1.49 | 1.25 | 1.76 |
| Acebutolol | 0.48 | 0.65 | 0.66 | 0.69 | 0.90 | 0.79 |
| Toliprolol | 0.58 | 1.54 | 1.12 | 0.94 | 1.68 | 1.91 |
| Oxprenolol | 0.72 | 1.54 | 1.25 | 1.33 | 1.57 | 1.90 |
| Bupranolol | 1.25 | 2.51 | 1.86 | 1.55 | 2.48 | 2.62 |
| Alprenolol | 1.34 | 2.23 | 1.60 | 1.42 | 2.36 | 2.43 |
| Propranolol | 1.49 | 2.62 | 2.00 | 1.52 | 3.03 | 2.72 |

[a] The maximum R. S. D. was ±11%. In most cases The R. S. D. was < ±3.8%.

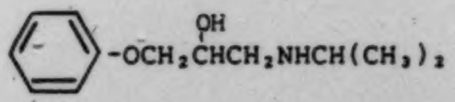
I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:CHOL:DCP (7:2:1 mole ratio)
V -- DMPC:DCP (7:1 mole ratio)

Table 8

Hydrophobic Substituent Constant of Functional Groups on the Aromatic Ring Structure of Beta Blockers at pH 7.4 and 30° C.[a]

| | Liposomes >Tc | | n-Octanol-buffer | |
|---|---|---|---|---|
| Functional Group | Log $K_{mx}$ | $\pi_x$ | Log $K_{mx}$ | $\pi_x$ |
| - | 3.05(H) | - | 2.11(H) | - |
| $-CH_3$(meta)(TPL) | 3.69 | 0.64 | 2.57 | 0.46 |
| $-CH_2CH{=}CH_2$(ortho)(APL) | 4.47 | 1.42 | 3.34 | 1.23 |
| $-OCH_2CH{=}CH_2$(ortho)(OPL) | 3.61 | 0.56 | 2.62 | 0.51 |
| $-CH_2CH_2OCH_3$(para)(MPL) | 3.43 | 0.38 | 2.28 | 0.17 |
| $-CH_2\underset{\text{O}}{\overset{}{\underset{\Vert}{C}}}NH_2$(para)(ATL) | 3.18 | 0.13 | 1.95 | -0.16 |
|  (PPL) | 4.73 | 1.68 | 3.37 | 1.26 |
|  (PDL) | 2.82 | -0.23 | 1.56 | -0.55 |

[a]determined from $\pi_x = \log K_{mx} - \log K_{mH}$ where $K_{mH}$ is the partition coefficient of pre-prenalterol



$-OCH_2\overset{OH}{C}HCH_2NHCH(CH_3)_2$

and $K_{mx}$ is the value for the derivative.

## E. THERMODYNAMIC ANALYSES

### The n-Octanol-Buffer System:

Thermodynamic parameters of the beta blockers in the n-octanol-buffer system are listed in Table 9. The magnitudes of $\Delta G^0_{w \rightarrow 0}$ are indicative of the spontaneity of the partition process and for the various beta blockers these are seen to be proportional to the degree of lipophilicity. Both $\Delta H^0_{w \rightarrow 0}$ and $\Delta S^0_{w \rightarrow 0}$ values are positive and except for ATL and PDL, each is approximately of equal magnitude among the drugs. Also, positive enthalpies and entropies of partitioning have been found in the phenol-cyclohexane (117) and the phenothiazine-n-octanol-aqueous phase systems (118). In contrast, negative enthalpies of partitioning of phenols in the n-octanol-saline system have been observed (165) indicating the presence of significant hydrogen bonding between molecules of phenol and n-octanol. Although many of the beta blockers are phenolic derivatives, their thermodynamic behavior does not resemble that of the phenols.

### Liposomes

Thermodynamic parameters of the beta blockers in DMPC liposomes are listed in Table 10. The free energies of partitioning in liposomes ($\Delta G^o_{W \rightarrow L}$ ) are slightly higher <Tc than >Tc of the phospholipid but the respective

# Table 9

The Thermodynamics of Partitioning of Beta Blockers in the n-Octanol-Phosphate Buffer System at pH 7.4 and 30° C.[a]

| Beta Blocker | $\Delta G^\circ_{w \to 0}$ $(KJmol^{-1})$ | $\Delta H^\circ_{w \to 0}$ $(KJmol^{-1})$ | $\Delta S^\circ_{w \to 0}$ $(Jmol^{-1}K^{-1})$ |
|---|---|---|---|
| Propranolol | -19.6 | 33.2 | 174 |
| Alprenolol | -19.4 | 42.5 | 204 |
| Bupranolol | -18.9 | 38.3 | 189 |
| Oxprenolol | -15.2 | 35.1 | 166 |
| Toliprolol | -14.9 | 39.8 | 180 |
| Acebutolol | -14.1 | 52.6 | 220 |
| Metoprolol | -13.2 | 44.1 | 189 |
| Atenolol | -11.3 | 19.3 | 101 |
| Pindolol | -9.1 | 11.1 | 66.6 |
| Nadolol | 0 | 0 | 0 |

[a] The maximum R. S. D. was ±5.3%.

Table 10

The Thermodynamics of Partitioning of Beta Blockers <Tc (15° C) and >Tc (30° C) in DMPC Liposomes[a]

| Beta Blocker | Below Tc (15° C) $\Delta G^\circ_{w\to L}$ (KJmol$^{-1}$) | $\Delta H^\circ_{w\to L}$ (KJmol$^{-1}$) | $\Delta S^\circ_{w\to L}$ (Jmol$^{-1}$K$^{-1}$) | Above Tc (30° C) $\Delta G^\circ_{w\to L}$ (KJmol$^{-1}$) | $\Delta H^\circ_{w\to L}$ (KJmol$^{-1}$) | $\Delta S^\circ_{w\to L}$ (Jmol$^{-1}$K$^{-1}$) |
|---|---|---|---|---|---|---|
| Propranolol | -22.8 | -11.2 | 40.8 | -27.4 | -6.6 | 68.6 |
| Alprenolol | -22.7 | 4.9 | 95.8 | -25.9 | 1.9 | 91.7 |
| Bupranolol | -23.3 | -6.4 | 58.8 | -27.2 | 8.3 | 117 |
| Oxprenolol | -17.2 | -30.1 | -44.8 | -21.0 | 10.6 | 104 |
| Toliprolol | -18.0 | -49.3 | -109.0 | -21.4 | 9.9 | 103 |
| Acebutolol | -10.6 | -172.0 | -562.0 | -17.0 | 13.4 | 100 |
| Metoprolol | -17.2 | 28.6 | -39.6 | -20.0 | 28.9 | 161 |
| Atenolol | -12.6 | -207.0 | -675.0 | -18.4 | 19.6 | 126 |
| Pindolol | -14.2 | -9.5 | -16.2 | -16.4 | 21.3 | 124 |
| Nadolol | 0 | 0 | 0 | -18.7 | -78.0 | -196 |

[a] The maximum R. S. D. was ±4.6%.

contributions of $\Delta H^{o}_{W \rightarrow L}$ and $\Delta S^{o}_{W \rightarrow L}$ are opposite. In the liquid crystalline state of the liposomes (>Tc), enthalpies and entropies are greater (except APL) than in the gel state (<Tc). Nadolol partitions under strong enthalpy control only in fluid liposomes, probably arising from polar group interactions between NDL and the bilayer. This behavior is opposite to that of a series of phenols (31, 117) which are generally unionized at neutral pH. With phenols <Tc of DMPC liposomes, $\Delta H^{o}_{W \rightarrow L}$ and $\Delta S^{o}_{W \rightarrow L}$ were positive and >Tc were negative. Enthalpies and entropies of partitioning of ionized phenothiazines in DMPC liposomes were found to be positive both >Tc and <Tc (118).

The enthalpies and entropies of partitioning are greater in the n-octanol-buffer system than in liposomes >Tc for 7 beta blockers listed in Table 9 and 10. For example, the ratio of $\Delta H^{o}_{W \rightarrow O}$ (n-octanol) : $\Delta H^{o}_{W \rightarrow L}$ (liposomes) is approximately 4:1 (on average), the same was found for phenothiazines (118). In contrast, this same ratio is 80:1 <Tc of DMPC which compares with 1.3:1 for phenothiazines (118). These results suggest a different mechanism of interaction of beta blockers with model membranes than the phenothiazines.

## Solution Calorimetry

The change in enthalpy of solution of propranolol in phosphate buffer at pH 5.0 ($\Delta H^{o}_{W}$) and of propranolol in buffer-saturated n-octanol ($\Delta H^{o}_{O}$), and the enthalpy of

Table 11

Enthalpy of Transfer of Propranolol ($\Delta H^\circ_{w \to o}$) Determined from Enthalpies of Solution in pH 5.0 Phosphate Buffer ($\Delta H^\circ_w$ ) and in Buffer-Saturated n-Octanol ($\Delta H^\circ_o$ ) at 25° C.[a]

| Expt. # | $\Delta H^\circ_w$ (KJmol$^{-1}$) | $\Delta H^\circ_o$ (KJmol$^{-1}$) | $\Delta H^\circ_{w \to o}$ (KJmol$^{-1}$) |
|---|---|---|---|
| 1 | -5.31 | 20.62 | 25.93 |
| 2 | -4.65 | 19.72 | 24.37 |
| 3 | -5.49 | 22.21 | 27.70 |
| Mean±S.D. | 5.15±0.44 | 20.85±1.26 | 26.00±1.67 |

---

[a] 100 mg of propranolol free base and 100 g solvent was used.

transfer of propranolol from the aqueous buffer to the n-octanol phase ($\Delta H^{o}_{W-O}$) are given in Table 11. The mean value of $\Delta H^{\circ}_{W}$ was -5.15 having a relative standard deviation of 8.5%. The negative sign indicates that the process underwent an exothermic reaction. The mean values of $\Delta H^{\circ}_{O}$ was 20.85 and endothermic having a relative standard deviation of ±6%. The $\Delta H^{\circ}_{W-O}$ was determined using equation 17, and had a mean value of 26.00±6.4% S.D. indicating that overall the transfer process was endothermic.

## F. INTERACTIONS OF PROPRANOLOL WITH MODEL AND BIOLOGICAL MEMBRANES

### Uptake Studies

The comparative uptakes of PPL by human erythrocytes, erythrocyte ghosts and liposomes after equilibration with initial concentrations of PPL varying from 0.05 to 2.3 mM are described in Figure 10. It can be seen that the uptake in neutral DMPC liposomes is linear with respect to PPL concentration and considerably less than that found with erythrocyte or erythrocyte ghost cells above 1 mM. It is noted, however, that uptake in cells is discontinuous over the concentration range. Initially, a linear uptake is observed which reaches a plateau at about 0.3 mM PPL, remains approximately constant to about 0.6 mM PPL then the uptake again rises linearly, but at a rate somewhat less than the initial rate. These data suggest that as the PPL

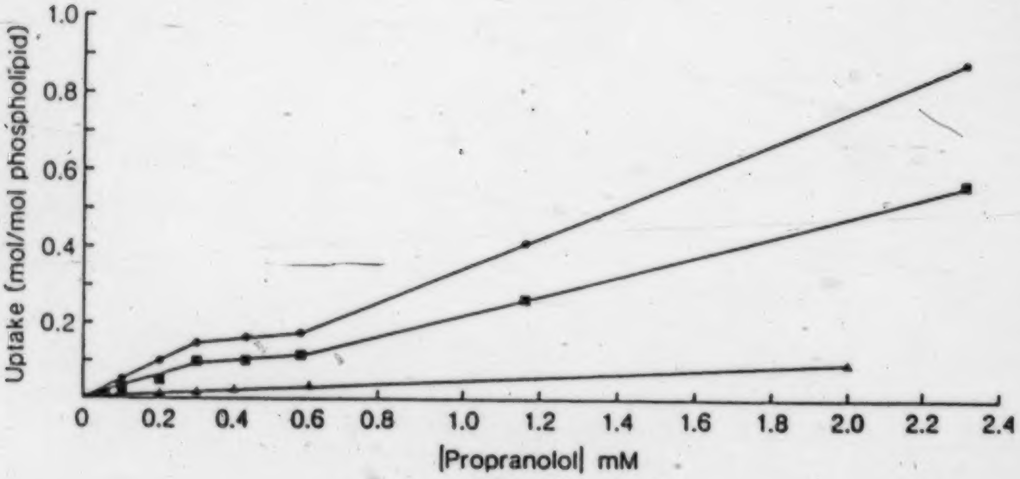


Figure 10. Uptake of PPL as a function of the initial PPL concentration. ● erythrocytes; ■ ghosts; ▲ neutral (DMPC) liposomes at pH 7.4. (The maximum R. S. D. was ±4%. The range of quadruplicate experimental values does not extend beyond the symbol dimensions in all cases)

concentration is increased the erythrocyte or ghost membrane undergoes a molecular conformational rearrangement, perhaps leading to a phase change in the membrane, which confers a different degree of affinity for PPL. This type of behavior does not occur in liposomes indicating that perhaps interaction of PPL with the phospholipid backbone of the erythrocyte cell membrane is mediated by other membrane components, such as protein (166) or other types of lipids including acidic phospholipids (167, 168) giving rise to a complex and causes the not understood re-organization of the cell membrane. Uptake of PPL in erythrocytes at three temperatures (25°, 30° and 37° C) was found to be only slightly temperature dependent as shown in Figure 11, but with retention of the apparent concentration-dependent phase change. Thus, the uptake appears to be controlled by the physical properties of the liquid crystalline state of the membrane itself on which PPL exerts a profound influence.

The interaction of PPL with neutral liposomes can be quantitated by applying a partition model (167). However, when interaction occurs via negatively-charged polar surface groups, such as with erythrocytes or ghosts, an adsorption model may be more appropriate. Figure 12 clearly shows that the uptake of PPL per g of dry membrane rapidly decreases as the weight of membrane is increased, then gradually levels off. Similar partitioning behavior in neutral liposomes has been observed (169). Consequently, a comparison of $K'_m$ in erythrocytes, ghosts and liposomes has been made.

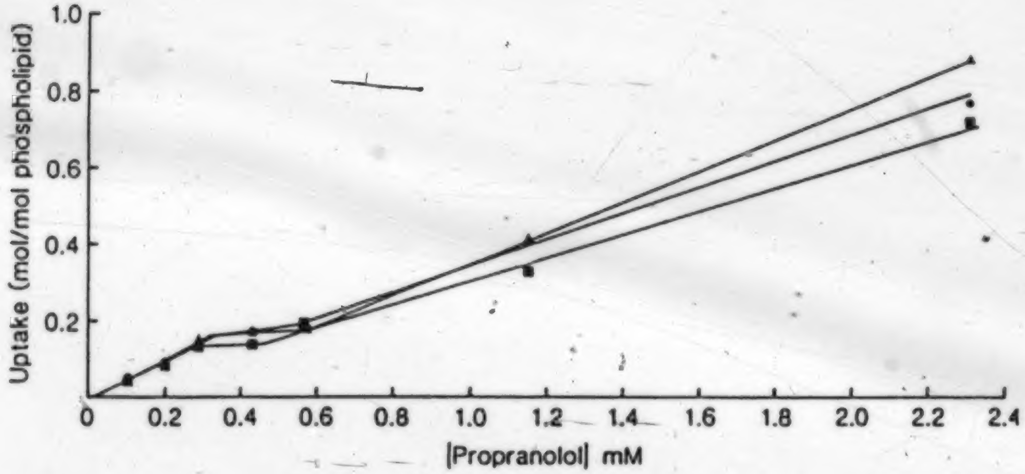


Figure 11. Effect of temperature on the uptake of PPL by erythrocytes. ● 25° C; ■ 30° C; ▲ 37° C. (The maximum R. S. D. was ±3%. The range of quadruplicate experimental values does not extend beyond the symbol dimensions in all cases)

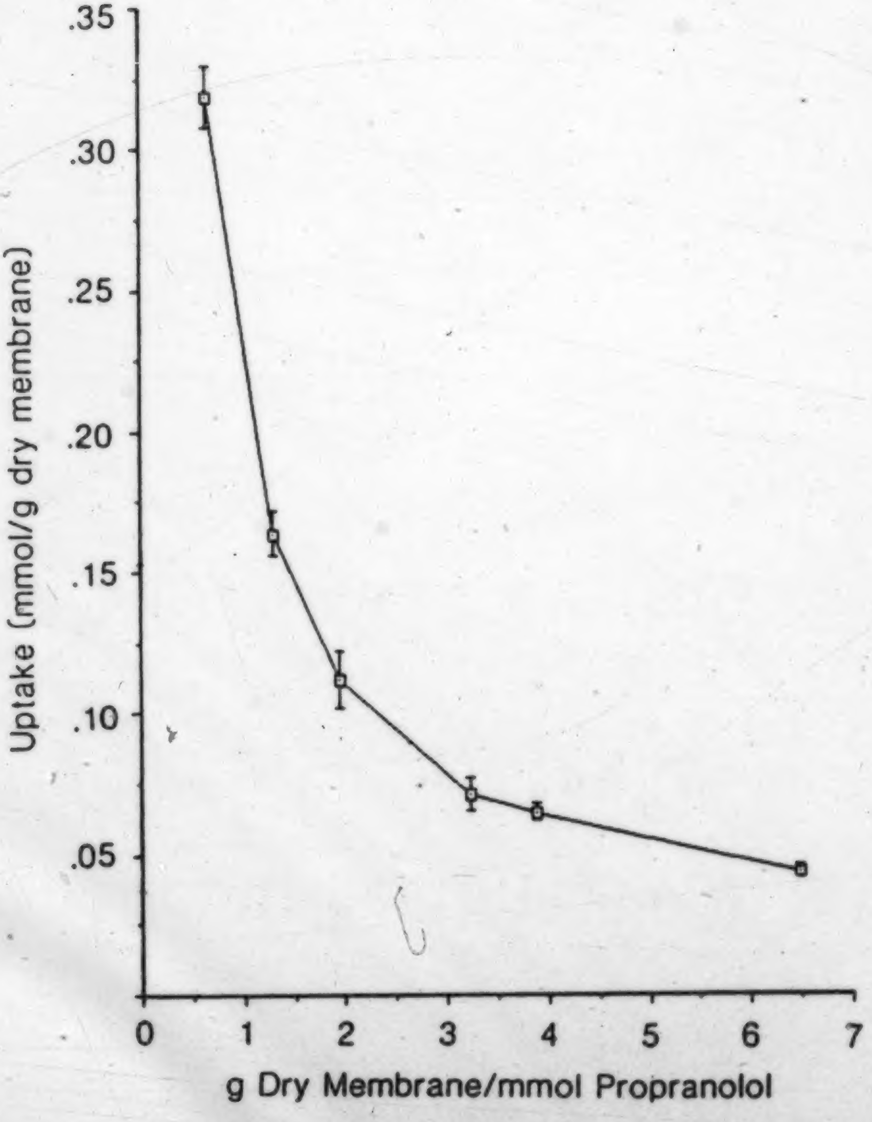


Figure 12. Efficiency of uptake of PPL in ghosts. Test samples were prepared by varying the volumes of stock ghost suspension and stock PPL solution.

**Partition Coefficients**

The log $K_m$ of PPL as a function of the initial PPL concentration in each of the three membrane systems is graphically illustrated in Figure 13. Log $K_m$ appears to remain fairly constant in the erythrocyte cell systems but it undergoes an intial decrease up to about 0.6 mM PPL before becoming approximately constant in liposomes. Similar behavior has been reported by others for membrane/buffer partition coefficients of alcohols (163) and phenols (164). The log $K$ in erythrocytes and ghosts is greater than that in neutral liposomes similar to the observed uptake but lower values of log $K_m$ in erythrocytes compared to ghosts were obtained because of the greater membrane dry weight of the former, likely due to proteins and other molecules which are normally part of the erythrocyte membrane.

The temperature-dependency of log $K'_m$ in ghosts and liposomes is depicted in Figure 14. The dramatic change in the log $K'_m$ as the temperature is increased through the Tc of DMPC (23° C) is clearly evident (59), whereas the change is barely discernible in the ghost membranes. This is understandable on the basis of earlier arguments with respect to the uptake results and, also with the knowledge that the Tc of individual phospholipids in mixed membranes is often obscured or eliminated (170). The indication that a break occurs in the slope of the curve is not meant to suggest that there is necessarily a change in the fluidity of the membrane but, it implies only that in this

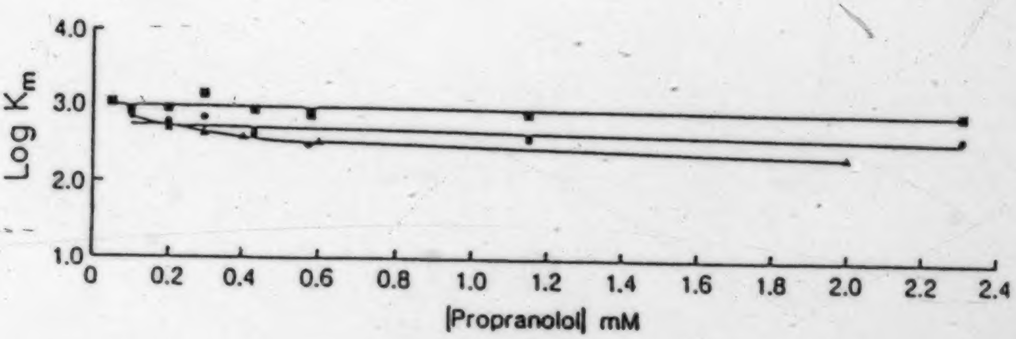


Figure 13. Variation of log K with initial PPL concentration. ● erythrocytes; ■ ghosts; ▲ neutral liposomes. Values of K are expressed as mole (Kg. dry membrane)$^{-1}$/mole $l^{-1}$. (The maximum R. S. D. was ±1.9%. The range of experimental values does not extend beyond the symbol dimensions in all cases)

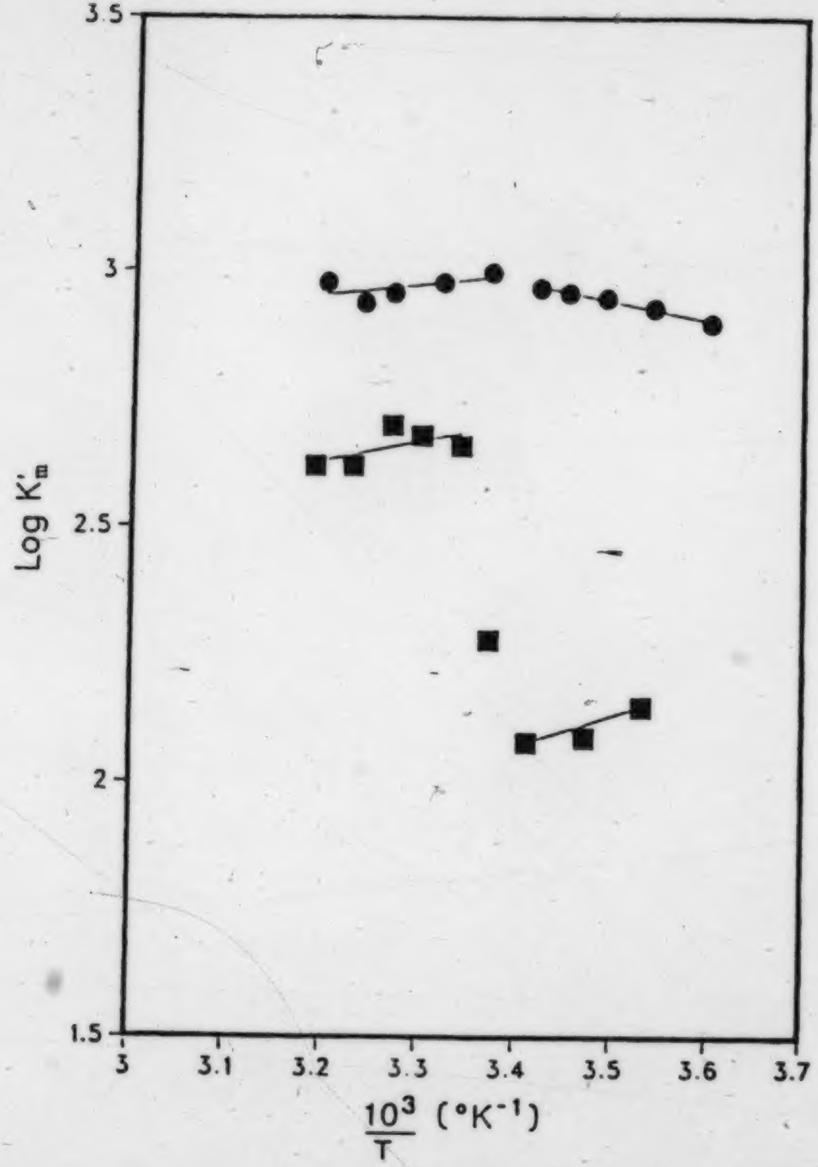


Figure 14. Van't Hoff plots of K'$_m$ as a function of temperature. ● erythrocyte ghosts; ■ neutral DMPC liposomes at pH 7.4. (The maximum R. S. D. was ±2.4%. The range of experimental values does not extend beyond the symbol dimensions in all cases)

temperature region a new organizational phenomenon begins to take place in the ghost membrane. Although its effect is almost negligible at the temperature seen at the break, it becomes increasingly important in a roughly exponential manner with decreasing temperature (170).

The energetics of the partitioning of PPL under these conditions, expressed as the free energy, $\Delta G^{o}_{W\rightarrow L}$, enthalpy $\Delta H^{o}_{W\rightarrow L}$, and entropy $\Delta S^{o}_{W\rightarrow L}$, determined at 37° C using standard equations (31) are given in Table 12. It is apparent that although the partitioning is spontaneous in each membrane system, i.e. $\Delta G^{o}_{W\rightarrow L}$ is negative, the energetics of individual processes differ. The transfer of PPL molecules to erythrocyte ghosts from the aqueous phase is accompanied by a net exchange of heat energy ($\Delta H^{o}_{W\rightarrow L}$) derived from the breaking of electrostatic and hydrogen bonds with water and the reformation of attractive bonds with the phospholipids in the erythocyte ghost membranes. The overall negative $\Delta H^{o}_{W\rightarrow L}$ indicates that the interactions involving PPL is stronger in the membranes than in the aqueous phase resulting in a net loss of heat in the exchange. Furthermore, the transfer of PPL from the aqueous phase to the membrane results in a net positive $\Delta S^{o}_{W\rightarrow L}$, although PPL molecules would have transferred into a more restricted environment. Hence, this leads to the conclusion that the ghost membrane molecules have undergone considerable disruption in their ordered states as a result of accommodating PPL molecules. In comparison, partitioning

Table 12

Thermodynmic Parameters for the Partitioning of Propranolol in Erythrocyte Ghosts and Liposome Membrane System at 37° C and pH 7.4[a].

| | $\Delta G^\circ_{W\rightarrow L}$ ($KJmol^{-1}$) | $\Delta H^\circ_{W\rightarrow L}$ ($KJmol^{-1}$) | $\Delta S^\circ_{W\rightarrow L}$ ($Jmol^{-1}K^{-1}$) |
|---|---|---|---|
| Erythrocyte ghosts | -17.72 | -2.66 | 48.56 |
| Liposomes | -15.53 | -6.66 | 28.61 |

[a] The maximum R. S. D. was ±3.2%.

of PPL in DMPC liposomes is driven by a lower positive $\Delta S^{o}_{W \rightarrow L}$ but which is sufficient to overcome a greater net negative $\Delta H^{o}_{W \rightarrow L}$ indicating that the accommodation of PPL molecules in the DMPC bilayers causes some disruption of the ordered state of the phospholipid bilayer but not accompanied by any strong polar group interaction between phospholipid and drug molecules, in contrast to that observed for acidic phospholipid liposomes (167, 171).

## Protection Against Osmotic Hemolysis

One of the actions of PPL that is claimed to be related to its membrane-stabilizing properties is protection of erythrocytes against hypotonic hemolysis (172). Figure 15 shows the relative hemolysis of erythrocytes over a fairly wide range of PPL concentration. It is apparent that there is gradual reduction of the percent relative hemolysis over two orders of magnitude of PPL concentration until about 1 mM, it remains unchanged from 1 mM to about 2.4 mM, then the percent relative hemolysis increases rapidly as concentrations approach 10 mM. The maximum amount of protection obtained was approximately 54%. This compares with 37.6% found for steroids (173) and about 80% found for tert-butylphenol (164).

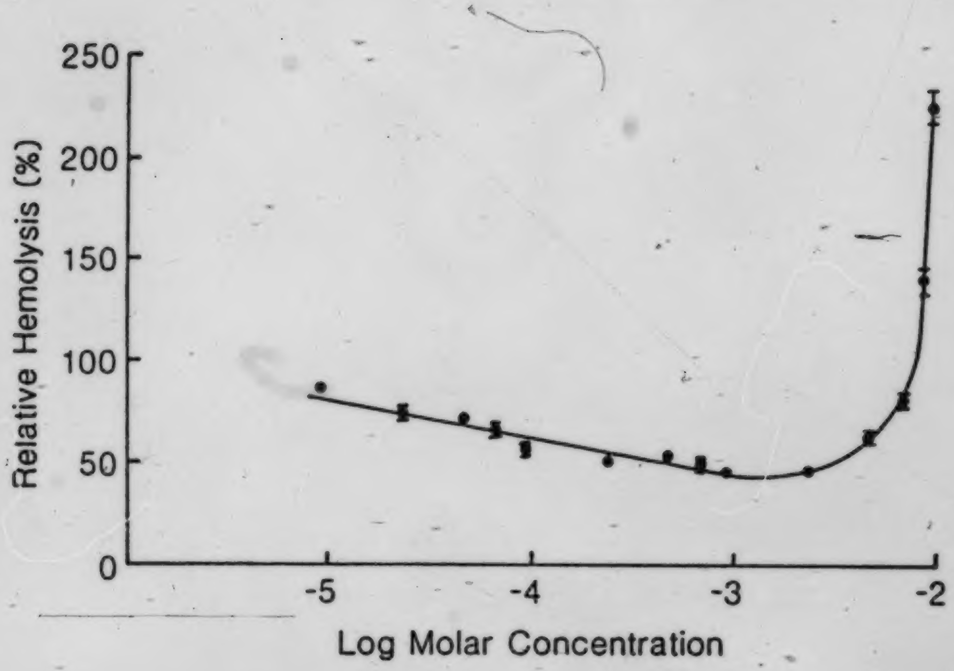


Figure 15. Effect of PPL concentration on the relative hemolysis of human erythrocytes.

## G. INTERACTIONS OF BETA BLOCKERS WITH SONICATED DMPC LIPOSOMES

Unilamellar liposomes prepared by sonication give rise to high resolution proton NMR spectra. The addition of lanthanide ion increases the chemical shift mainly of the external trimethylammonium group. The addition of $Eu^{3+}$ causes an upfield shift whereas $Pr^{3+}$ causes a downfield shift. Figure 16 is a proton NMR spectrum of DMPC. Figure 17 shows the spectra of a)DMPC b)PPL c)DMPC + PPL d)DMPC + $Pr^{3+}$ e)DMPC + PPL + $Pr^{3+}$. These spectra were plotted from 0-4 ppm because this range includes the peak of interest (trimethylammonium). Detailed spectra have been included in the appendix.

### Effect of $PrCl_3$

Increasing the concentration of $Pr^{3+}$ increased the downfield shift of the external peak. The difference in chemical shift (ΔHz) of the external trimethylammonium group and internal peaks (Δext-Δint) correlated with the $Pr^{3+}$ concentration (r=.99) as shown in Figure 18. Also broadening of the line width of the external peak occurred as the concentration of $Pr^{3+}$ was increased.

### Effect of Beta Blockers

Addition of the beta blockers PPL, APL, TPL, OPL, MPL and ATL to DMPC (14.4 mM) + $Pr^{3+}$ (2.5 mM) are shown in Figures 19 and in appendix. As seen in Figure 19, PPL

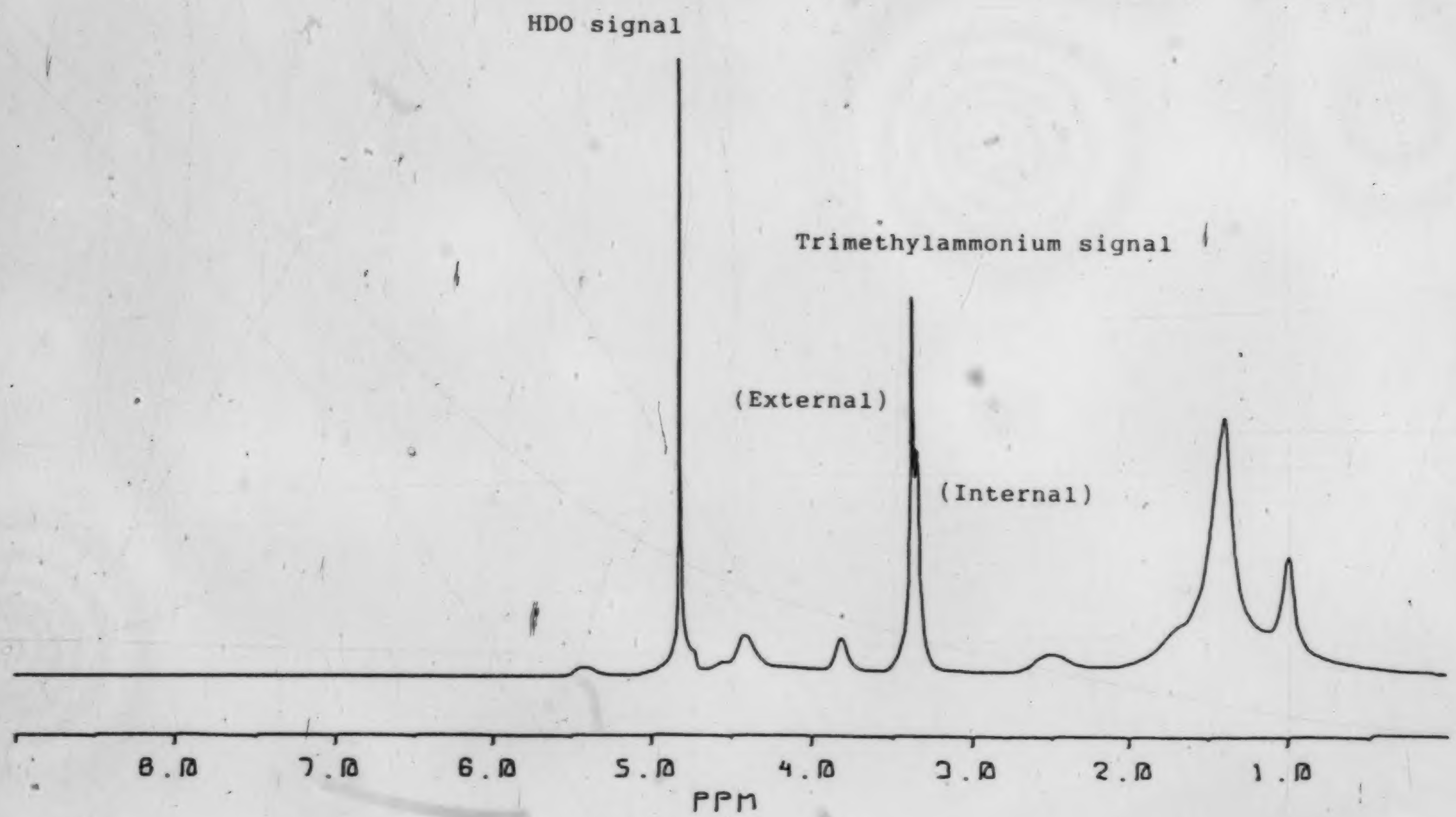


Figure 16. $^1$H-NMR spectrum of DMPC liposomes.

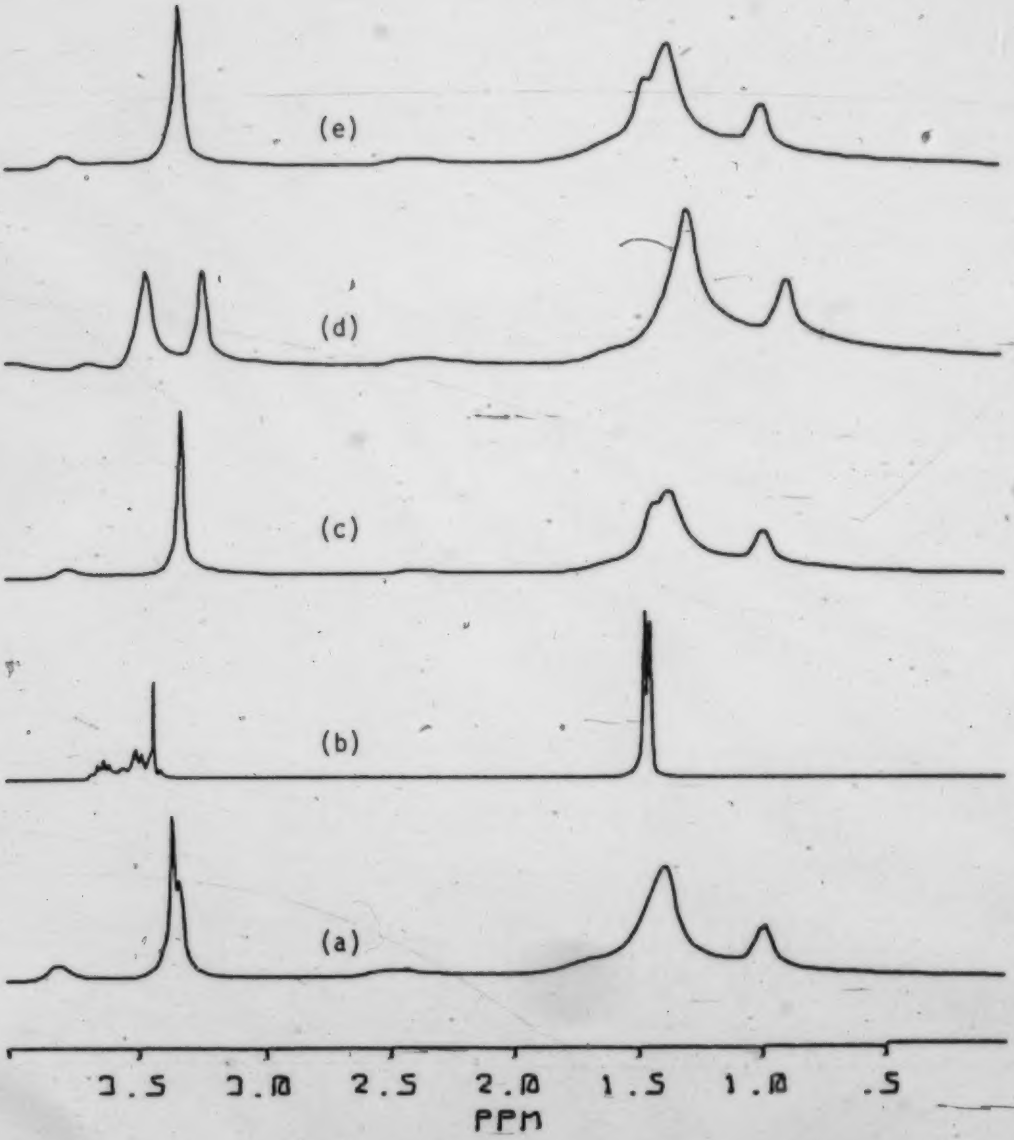


Figure 17. $^1$H-NMR spectra of liposomes of a)DMPC(14.4 mM); b)PPL(5 mM); c) DMPC(14.4 mM) + PPL(5 mM); d) DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) and e) DMPC(14.4 mM) + PPL(5 mM) + $Pr^{3+}$(2mM) from 0-4 ppm chemical shift.

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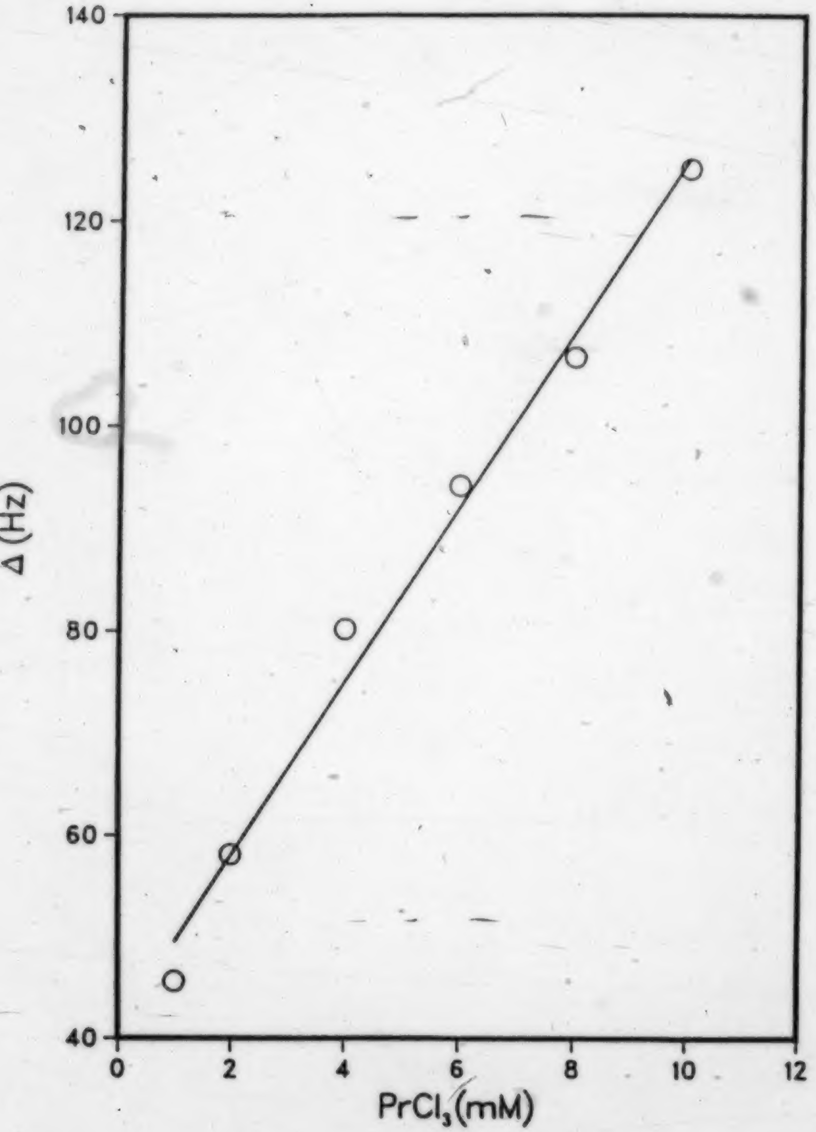


Figure 18. Correlation of difference in internal and external peak of choline (Δ) and $PrCl_3$ concentration.

reversed the effect of $Pr^{3+}$ completely at 5 mM. With other beta blockers higher concentrations were required to displace $Pr^{3+}$ from the membrane surfaces. In the case of OPL or TPL it was not able to completely reverse the effect of $Pr^{3+}$ even at concentrations as high as 20 mM. Figure 20 is a composite of the data from Figure 19 and figures from appendix with respect to the displacement of $Pr^{3+}$ from the membrane surfaces as a function of beta blocker concentration. Each of the beta blockers used in this study yielded, a linear relationship but having a different slope. The greater the slope, the better was the efficiency of the beta blocker to displace $Pr^{3+}$. A plot of the displacement constant (P) obtained from the slopes of Figure 20 versus log $K'_m$ of beta blockers is illustrated in Figure 21. Four of the beta blockers namely PPL, APL, TPL and OPL obeyed a linear relationship ($r=0.99$, $p<0.01$) whereas MPL and ATL did not. This is particular significant since beta blockers which conform to this linear relationship are nonselective whereas MPL and ATL are regarded as being cardioselective.

## H. QSAR OF BETA BLOCKERS

### Correlations of Partitioning Between the n-Octanol-Buffer System and Liposomes

Correlations of log K' or log K of 9 beta blockers in the n-octanol-buffer and log $K'_m$ or log $K_m$ in DMPC, DPPC, DMPC:CHOL, DMPC:DCP and DMPC:CHOL:DCP liposomes yielded

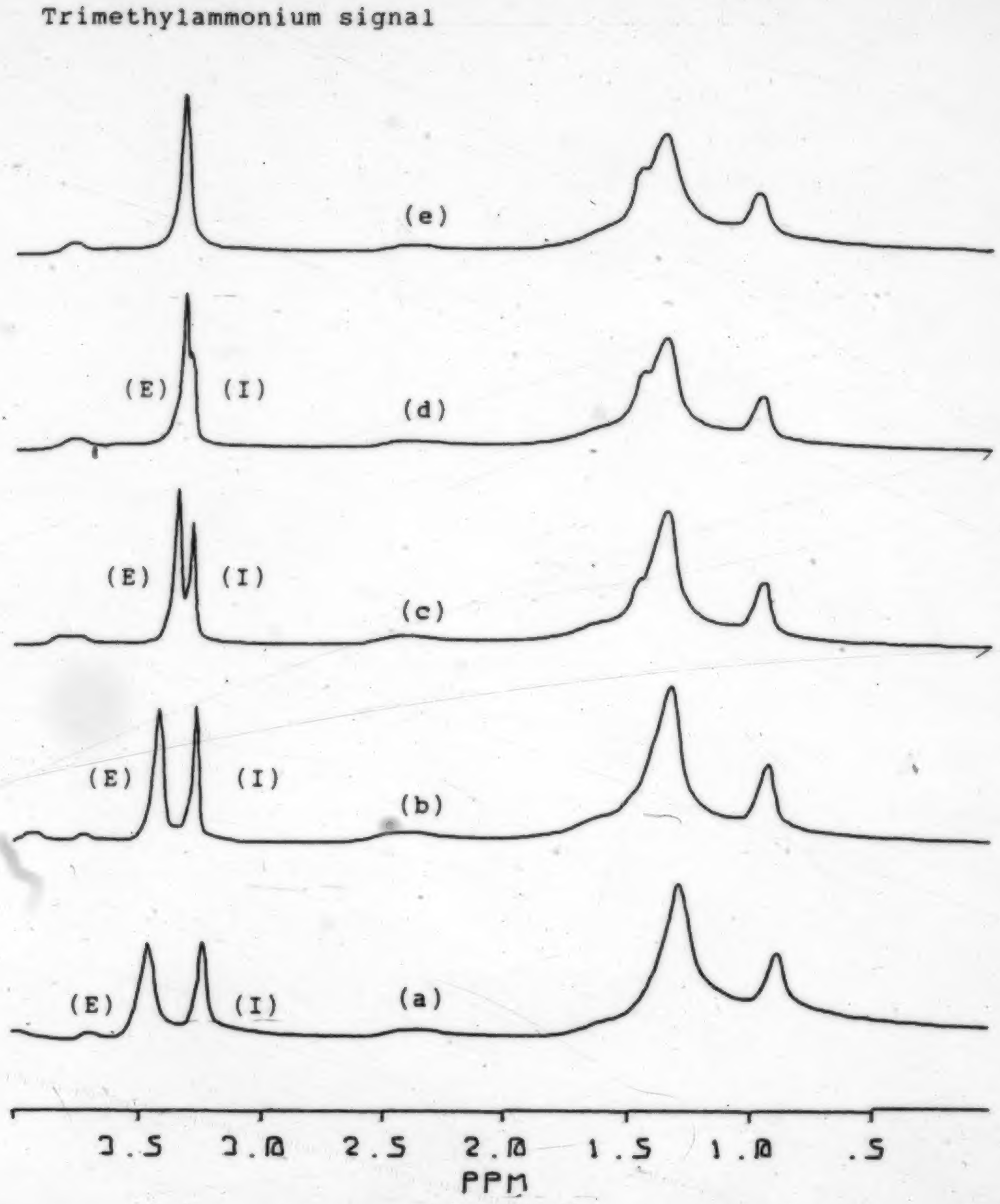


Figure 19. $^1$H-NMR spectra of liposomes of DMPC (14.4 mM) + Pr$^{3+}$ (2 mM) as a function of PPL: a)0 mM; b)0.5 mM; c)2 mM; d)4 mM and e)5 mM.

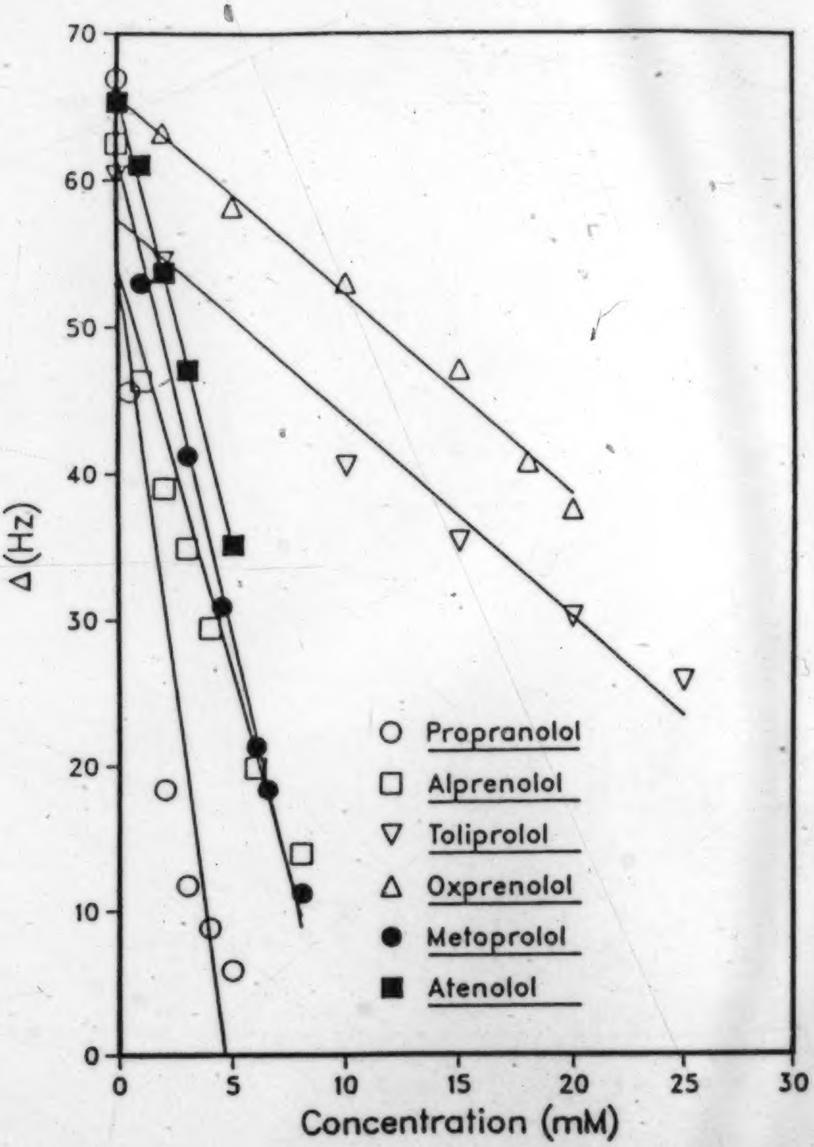


Figure 20. A plot of ΔHz (the difference in chemical shift of the internal and external signals of the trimethylammonium groups of the DMPC liposomes) versus beta blocker concentration.

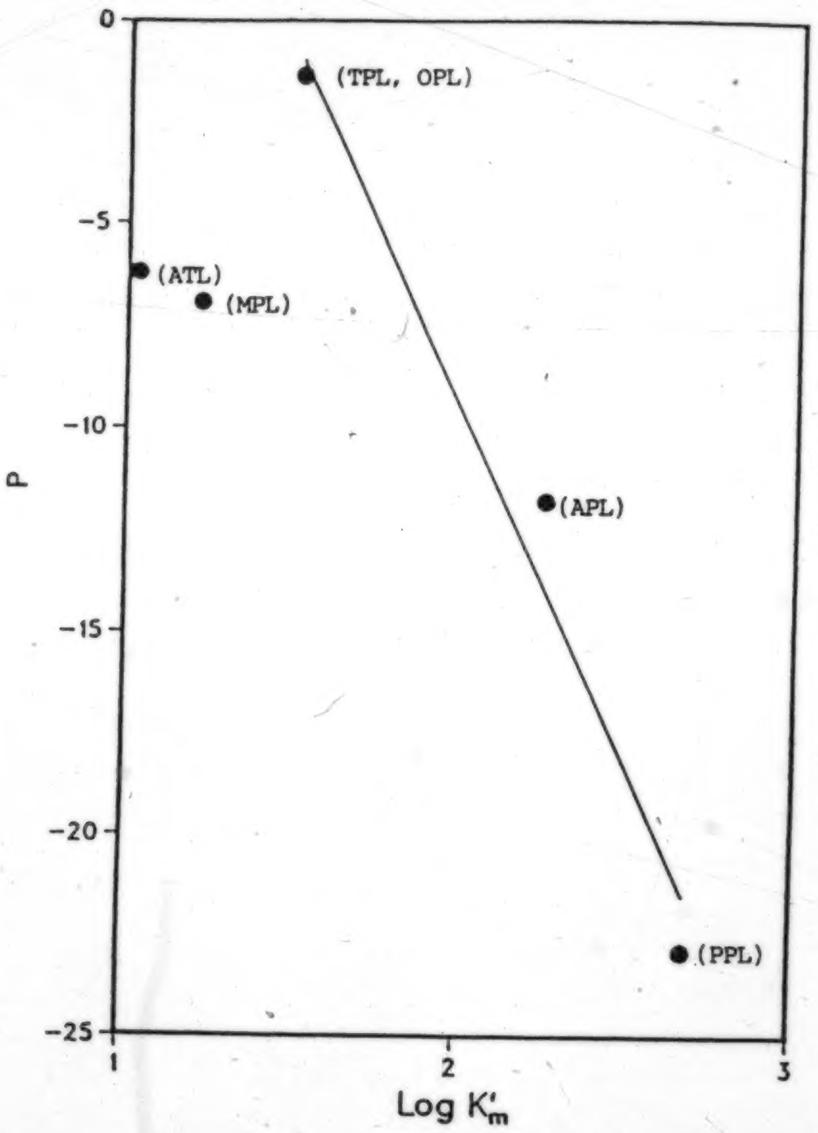


Figure 21. Plot of displacement constant (P) of $Pr^{3+}$ from DMPC liposomes by beta blockers versus their apparent partition coefficients.(log $K'_m$ )

linear relationships. The linear regression parameters are given in Table 13. Log K (n-octanol) vs log $K_m$ (liposomes) are shown in Figures 22-26. Correlation coefficients (r) of log K' vs log $K'_m$ ranged from 0.75 to 0.94 and from 0.83 to 0.94 for plots of log K and log $K_m$. Correlations were statistically significant either at $p<0.05$ or $p<0.01$. The best correlation was found with liposomes of DMPC:CHOL:DCP (7:2:1 mole ratio) as shown in Figure 26 or Table 13.

## Correlations Between Partition Coefficients in the n-Octanol-Buffer System and Pharmacokinetic Parameters

Pharmacokinetic parameters of beta blockers (174) in humans are listed in Table 14. Correlations between K' or K and pharmacokinetic parameters were calculated and linear regression parameters determined (Table 15). When correlations were calculated using K' 6 out of 7 parameters were correlated significantly at $p<0.05$. However when K values were used only 1 out of 7 parameters was significant at $p<0.05$. The ratio of the fraction of drug bound and unbound to $\alpha_1$-acid glycoprotein did not correlate with log K' or log K in the n-octanol-buffer system. A similar pattern of behavior has previously been reported for beta blockers in the n-octanol-buffer system (174).

Table 13

Linear Regression Parameters Derived from Correlations of log K' or log K and log $K'_m$ or log $K_m$ of 9 Beta Blockers

| Liposome Composition* | a | b | r | p |
|---|---|---|---|---|
| | Log K' | | | |
| Log $K'_m$ | | | | |
| DMPC | 0.99 | 0.99 | 0.85 | <0.01 |
| DPPC | 0.76 | 0.75 | 0.81 | <0.01 |
| DMPC:CHOL(1:1) | 0.73 | 0.57 | 0.75 | <0.05 |
| DMPC:DCP(7:1) | 0.85 | 1.34 | 0.80 | <0.01 |
| DMPC:CHOL:DCP(7:2:1) | 0.72 | 1.38 | 0.94 | <0.01 |
| | Log K | | | |
| Log $K_m$ | | | | |
| DMPC | 0.90 | 1.25 | 0.87 | <0.01 |
| DPPC | 0.71 | 1.40 | 0.87 | <0.01 |
| DMPC:CHOL(1:1) | 0.72 | 1.22 | 0.83 | <0.01 |
| DMPC:DCP(7:1) | 0.83 | 1.71 | 0.84 | <0.01 |
| DMPC:CHOL:DCP(7:2:1) | 1.23 | 0.34 | 0.94 | <0.01 |

* mole ratios in brackets

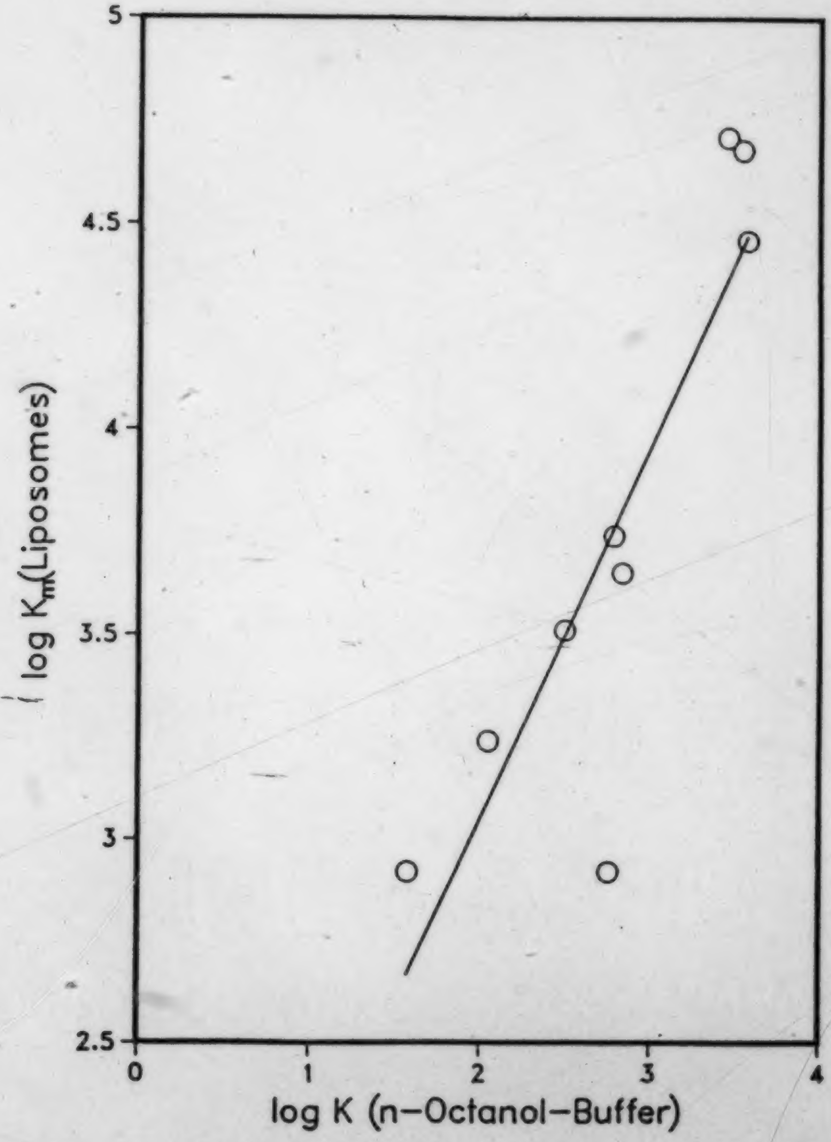


Figure 22. Correlation of the partition coefficients of beta blockers in the n-octanol-buffer system and DMPC liposomes at pH 7.4 and 37° C, (r=0.87).

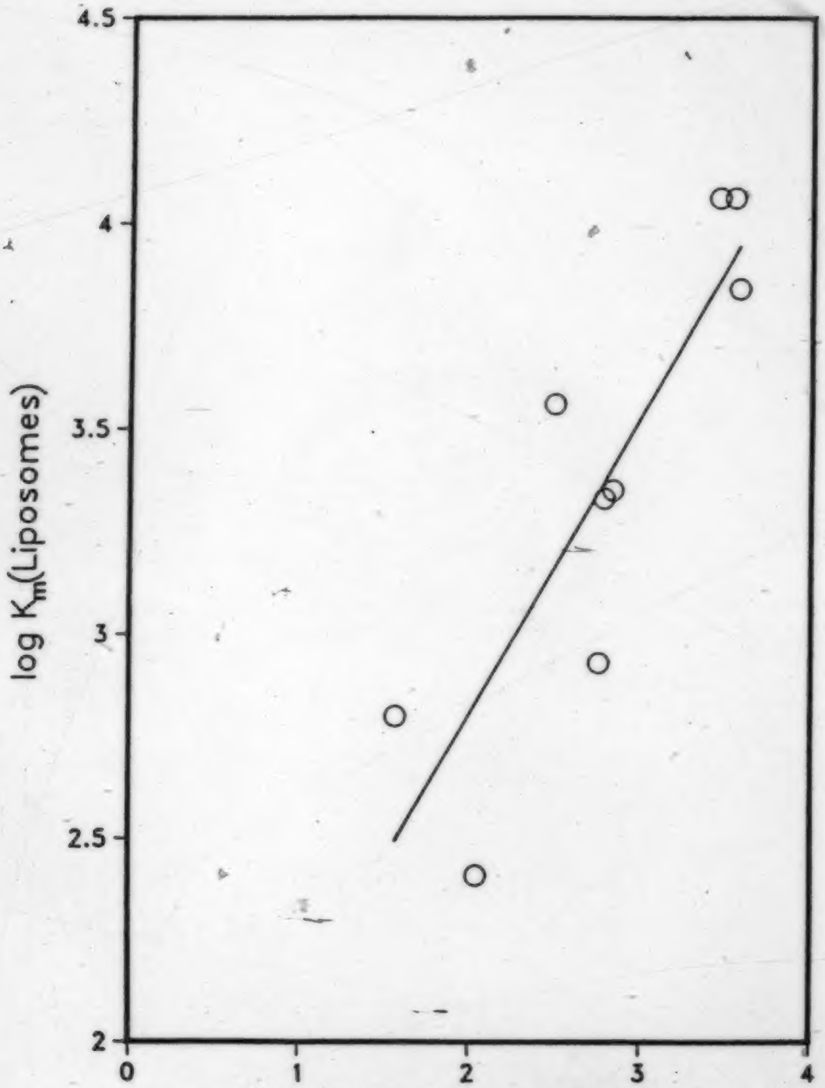


Figure 23. Correlation of the partition coefficients of beta blockers in the n-octanol-buffer system and DPPC liposomes at pH 7.4 and 37° C, (r=0.87).

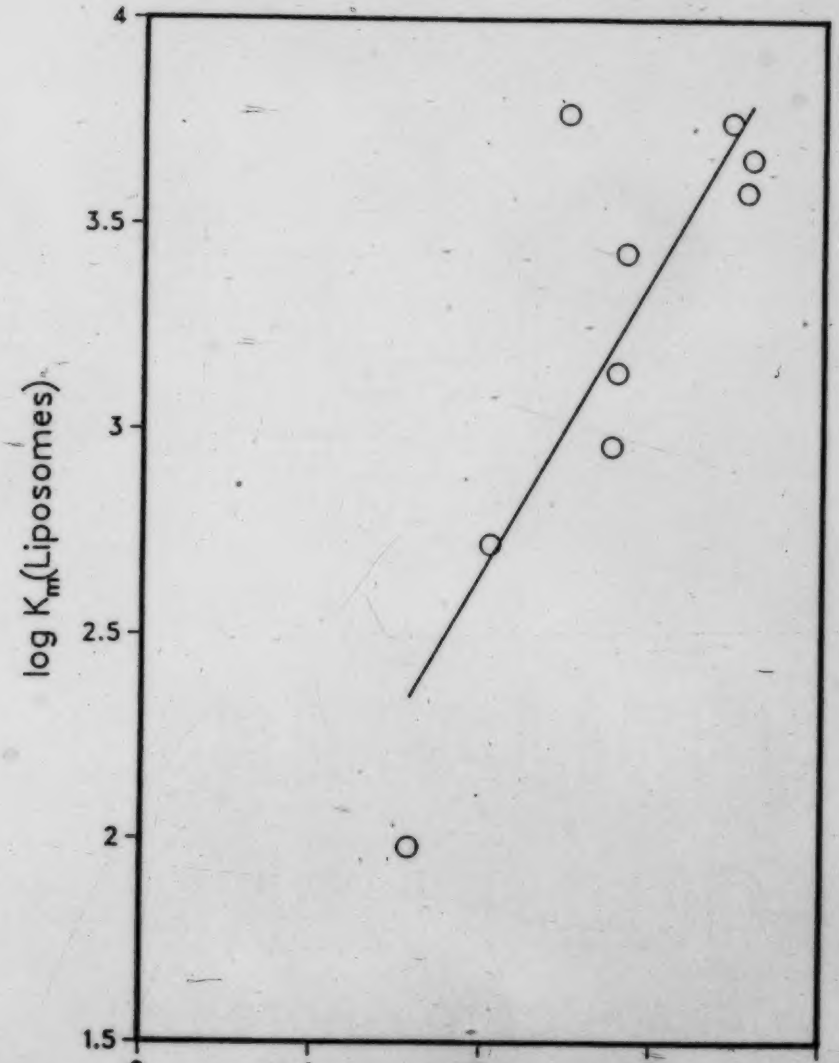


Figure 24. Correlation of the partition coefficients of beta blockers in the n-octanol-buffer system and DMPC: CHOL(1:1 mole ratio) liposomes at pH 7.4 and 37° C, (r=0.83).

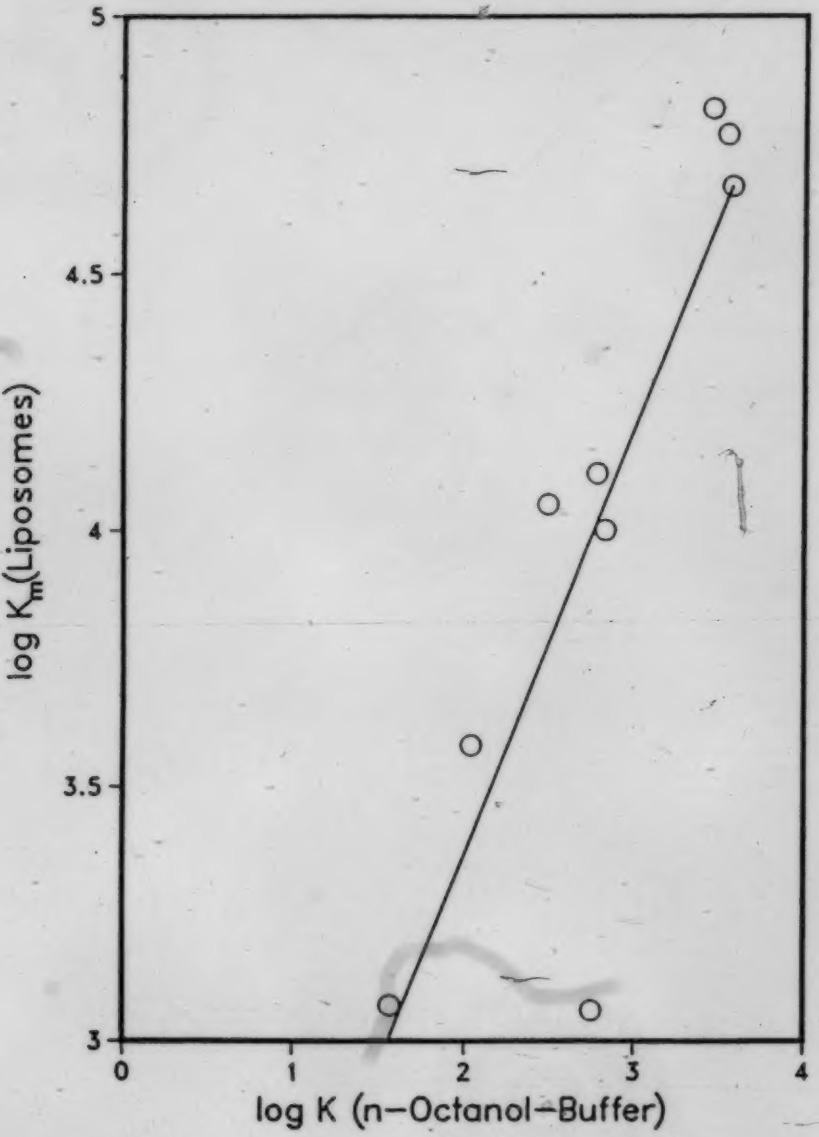


Figure 25. Correlation of the partition coefficients of beta blockers in the n-octanol-buffer system and DMPC: DCP(7:1 mole ratio) liposomes at pH 7.4 and 37° C, (r=0.84).

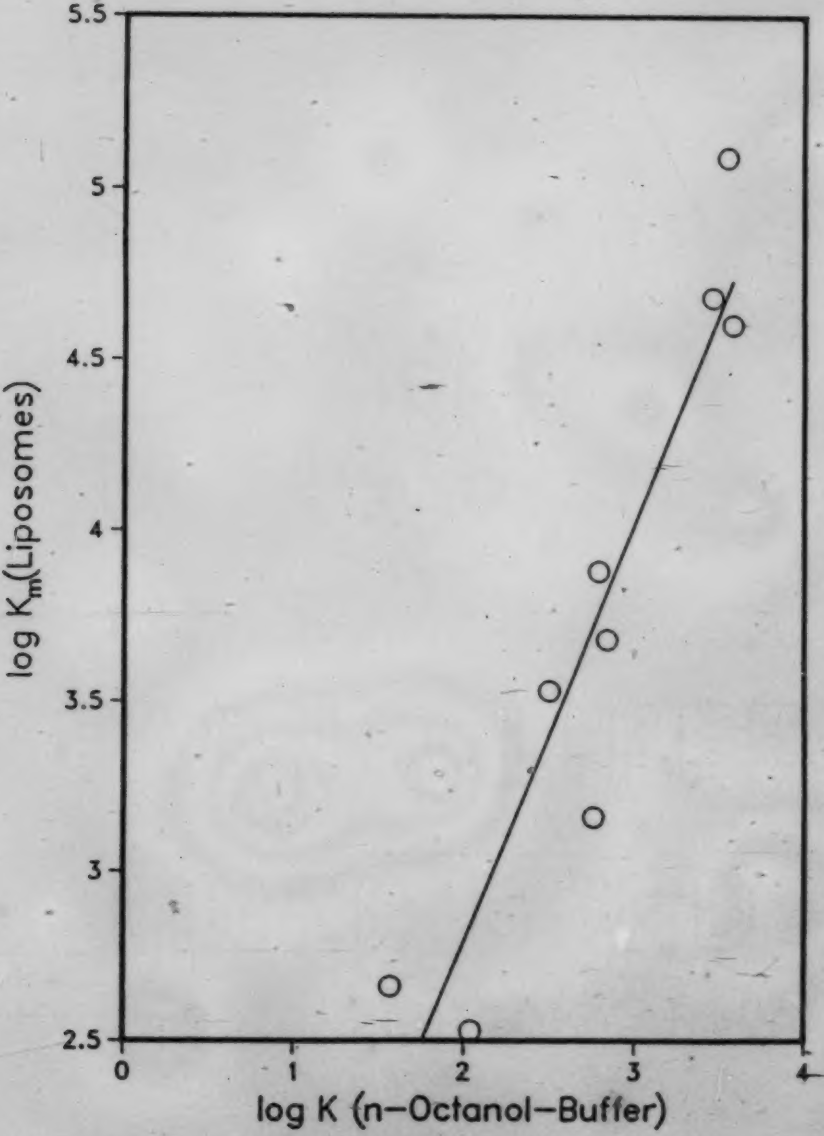


Figure 26. Correlation of the partition coefficients of beta blockers in the n-octanol-buffer system and DMPC: CHOL:DCP(7:2:1 mole ratio) liposomes at pH 7.4 and 37° C, (r=0.94).

Table 14

Mean Secondary Pharmacokinetic Parameters of Beta Blockers Determined in Humans*.

| Beta Blockers | log $K_p$ | log $r_A$ | log $r_G$ | log $K_{BC}$ | log Vuss | log $r_T$ | log r |
|---|---|---|---|---|---|---|---|
| Propranolol | 2.40 | 0.09 | 0.34 | 0.84 | 3.29 | 1.61 | 1.40 |
| Alprenolol | 1.78 | -0.18 | 0.08 | 0.67 | 2.50 | 0.91 | 1.82 |
| Oxprenolol | 2.34 | -0.53 | 0.41 | -0.004 | - | - | 0.91 |
| Acebutolol | 0.82 | - | - | 0.49 | 2.10 | 0.66 | 0.16 |
| Metoprolol | 1.12 | -0.81 | -1.18 | 0.00 | 2.35 | 0.88 | 0.75 |
| Pindolol | 1.44 | -0.65 | -0.35 | 0.22 | 2.30 | 0.46 | 0.00 |
| Atenolol | -0.23 | - | - | -0.14 | 1.90 | 0.20 | -1.20 |
| Nadolol | 0.87 | - | - | - | 2.27 | 0.56 | -0.50 |

* Ref. 174

$K_p$ - Partition coefficient of drug between plasma protein and plasma water
$r_A$ - Ratio of the fraction of drug bound and unbound to albumin
$r_G$ - Ratio of the fraction of drug bound and unbound to $\alpha_1$ - acid glycoprotein
$K_{BC}$ - True red blood cell partition coefficient
Vuss- Steady state volume of distribution referenced to the unbound drug in plasma
$r_T$ - Ratio of the fraction of drug bound and unbound to tissue
r - Ratio of the fraction of drug nonrenally and renally eliminated

Table 15

Linear Regression Parameters Derived from Correlations of log K' or log K and Pharmacokinetic Parameters of Beta Blockers.

| Pharmacokinetic Parameter* | a | b | r | p |
|---|---|---|---|---|
| | Log K' | | | |
| $\log K_p$ | 1.31 | 0.70 | 0.76 | $<0.05 (n=7)$ |
| $\log r_A$ | 0.54 | -0.83 | 0.95 | $<0.05 (n=5)$ |
| $\log r_G$ | 0.72 | -0.69 | 0.70 | - |
| $\log K_{BC}$ | 0.51 | -0.01 | 0.84 | $<0.05 (n=7)$ |
| log Vuss | 0.58 | 2.08 | 0.81 | $<0.05 (n=7)$ |
| $\log r_T$ | 0.61 | 0.43 | 0.85 | $<0.05 (n=6)$ |
| log r | 1.40 | -0.29 | 0.87 | $<0.05 (n=7)$ |
| | Log K | | | |
| $\log K_p$ | 0.73 | -0.57 | 0.58 | - |
| $\log r_A$ | 0.35 | -0.39 | 0.79 | - |
| $\log r_G$ | 0.41 | -1.28 | 0.52 | - |
| $\log K_{BC}$ | 0.37 | -0.69 | 0.72 | - |
| log Vuss | 0.40 | 1.36 | 0.66 | - |
| $\log r_T$ | 0.48 | -0.48 | 0.79 | - |
| log r | 1.11 | -2.42 | 0,81 | $<0.05 (n=7)$ |

* See Table 14

## Correlations Between Partition Coefficients in the n-Octanol-Buffer System and Corneal Penetration

The corneal penetration of beta blockers (175) in rabbits is summarized in Table 16. These have been correlated with log K' or log K of the beta blockers as given in Table 2. The linear regression parameters are shown in Table 17. However, ~~the~~ correlations were not statistically significant (r=0.68) in the n-octanol-buffer system.

## Correlations Between Partition Coefficients of Beta Blockers in Various Liposome Compositions and Corneal Penetration

Correlations of the corneal penetration of beta blockers (175) in rabbits and log $K'_m$ and log $K_m$ in various liposome compositions yielded the linear regression parameters presented in Table 17. Except for DMPC, the other liposome compositions produced statistically significant correlations using either log $K'_m$ or log $K_m$. However, a significant correlation with DMPC liposomes was found using log $K_m$ and the best correlation was found when DMPC:CHOL liposomes were employed.

## Correlations Between Partition Coefficients in DMPC Liposomes and Pharmacokinetic Parameters

Correlations between log $K'_m$ or log $K_m$ in DMPC liposomes <Tc (10° C) and >Tc (37° C) and pharmacokinetic parameters of beta blockers (Table 14) are summarized in Table 18

Table 16

Permeability Coefficients Across the Intact Excised Rabbit Cornea of Beta Blockers*.

| Beta Blocker | log $P_T$(cm/sec) |
|---|---|
| Propranolol | -4.24 |
| Oxprenolol | -4.56 |
| Metoprolol | -4.62 |
| Acebutolol | -6.07 |
| Nadolol | -5.99 |
| Atenolol | -6.17 |

* Ref. 175

Table 17

Linear Regression Parameters Derived from Correlations of log K' or log K and of log $K'_m$ and log $K_m$ in Liposomes of Various compositions and the Corneal Penetration of Beta Blockers (n=9).

| Partitioning system* | Log $P_T$ | | | |
|---|---|---|---|---|
| | a | b | r | p |
| **Log K' or Log $K'_m$** | | | | |
| n-Octonol- buffer | 1.02 | -5.70 | 0.68 | - |
| DMPC | 1.01 | -6.62 | 0.81 | - |
| DPPC | 1.37 | -6.68 | 0.94 | $<0.01$(n=6) |
| DMPC:CHOL(1:1) | 1.97 | -7.33 | 0.99 | $<0.01$(n=5) |
| DMPC:DCP(7:1) | 1.08 | -6.98 | 0.88 | $<0.05$(n=6) |
| DMPC:CHOL:DCP(7:2:1) | 0.80 | -6.35 | 0.82 | $<0.05$(n=6) |
| | Log $P_T$ | | | |
| **Log K or Log $K_m$** | | | | |
| n-Octanol-buffer | 1.13 | -8.21 | 0.68 | - |
| DMPC | 1.17 | -9.37 | 0.83 | $<0.05$(n=6) |
| DPPC | 1.46 | -9.97 | 0.92 | $<0.01$(n=6) |
| DMPC:CHOL(1:1) | 1.94 | -11.50 | 0.93 | $<0.05$(n=5) |
| DMPC:DCP(7:1) | 1.24 | -9.93 | 0.90 | $<0.05$(n=6) |
| DMPC:CHOL:DCP(7:2:1) | 0.85 | -8.27 | 0.81 | $<0.05$(n=6) |

* mole ratios in brackets

and 19. The results show that at 10° C, r ranged from 0.60-0.98 using log $K_m$ values but $r_A$($r=0.98$, $p<0.01$) and Vuss ($r=0.83$, $p<0.05$) were significantly correlated. However using log $K_m$ r ranged from 0.46-0.87 and none of the parameters was significantly correlated. On the other hand, at 37° C 5 out of 7 parameters were significantly correlated using log $K'_m$. $r_G$ and $K_{BC}$ being the exceptions. However, when $K_m$ was used only 4 out of 7 parameters were significantly correlated; the exceptions in this case being $K_p$, $r_G$ and $K_{BC}$. The correlation coefficients were higher using log $K'_m$ compared to using log $K_m$. The correlations were markedly improved when the liposomes were at 37° C and in the liquid crystalline phase. (Tc of DMPC=23° C).

## Effect of Liposome Composition on Correlation of log $K'_m$ and log $K_m$ of Beta Blockers with Pharmacokinetic Parameters

Correlations determined between log $K'_m$ and log $K_m$ in DPPC, DMPC:CHOL (1:1 mole ratio) DMPC:DCP (7:1 mole ratio) and DMPC:CHOL:DCP (7:2:1 mole ratio) liposomes and pharmacokinetic parameters yielded the corresponding linear regression parameters presented in Tables 20-23. In all instances the correlations were better using log $K'_m$. Thus the number of pharmacokinetic parameters which yielded a significant correlation of log $K'_m$ with the various liposome compositions was as follows: DMPC-5/7 ; DPPC-5/7 ; DMPC:CHOL (1:1 mole ratio)-2/7 ; DMPC:CHOL:DCP (7:2:1 mole ratio)-6/7.

Table 18

Linear Regression Parameters Derived from Correlations of log $K'_m$ (DMPC Liposomes) and Pharmacokinetic Parameters of Beta Blockers <Tc (10° C) and >Tc (37° C).

| Pharmacokinetic Parameter* | a | b | r | p |
|---|---|---|---|---|
| | | Log $K'_m$ (10° C) | | |
| log $K_p$ | 0.93 | 0.31 | 0.66 | - |
| log $r_A$ | 0.65 | -1.34 | 0.98 | <0.01(n=5) |
| log $r_G$ | 0.71 | -1.14 | 0.60 | - |
| log $K_{BC}$ | 0.34 | -0.10 | 0.60 | - |
| log Vuss | 0.56 | 1.76 | 0.83 | <0.05(n=6) |
| log $r_T$ | 0.48 | 0.22 | 0.71 | - |
| log r | 1.04 | -0.65 | 0.68 | - |
| | | log $K'_m$ (37° C) | | |
| log $K_p$ | 0.93 | -0.03 | 0.72 | <0.05(n=8) |
| log $r_A$ | 0.56 | -1.45 | 0.99 | <0.01(n=5) |
| log $r_G$ | 0.73 | -1.46 | 0.66 | - |
| log $K_{BC}$ | 0.35 | -0.25 | 0.64 | - |
| log Vuss | 0.51 | 1.66 | 0.84 | <0.05(n=7) |
| log $r_T$ | 0.47 | -0.08 | 0.76 | <0.05(n=7) |
| log r | 1.12 | -1.20 | 0.76 | <0.05(n=8) |

* See Table 14

Table 19

Linear Regression Parameters Derived from Correlations of log $K_m$ (DMPC Liposomes) and Pharmacokinetic Parameters of Beta Blockers <Tc (10° C) and >Tc (37° C).

| Pharmacokinetic Parameter* | a | b | r | p |
|---|---|---|---|---|
| | Log $K_m$ (10° C) | | | |
| log $K_p$ | 0.78 | -1.15 | 0.57 | - |
| log $r_A$ | 0.47 | -2.04 | 0.87 | - |
| log $r_G$ | 0.44 | -1.66 | 0.46 | - |
| log $K_{BC}$ | 0.33 | -0.77 | 0.59 | - |
| log Vuss | 0.51 | 0.76 | 0.78 | - |
| log $r_T$ | 0.50 | -0.85 | 0.77 | - |
| log r | 1.10 | -3.02 | 0.74 | - |
| | log $K_m$ (37° C) | | | |
| log $K_p$ | 0.83 | -1.63 | 0.65 | - |
| log $r_A$ | 0.45 | -2.15 | 0.89 | <0.05(n=5) |
| log $r_G$ | 0.47 | -1.93 | 0.52 | - |
| log $K_{BC}$ | 0.34 | -0.92 | 0.63 | - |
| log Vuss | 0.48 | 0.67 | 0.81 | <0.05(n=7) |
| log $r_T$ | 0.50 | -1.02 | 0.82 | <0.05(n=5) |
| log r | 1.17 | -3.75 | 0.80 | <0.05(n=8) |

* See Table 14

Table 20

Linear Regression Parameters Derived from Correlations of log $K_m'$ and log $K_m$ (DPPC Liposomes) and Pharmacokinetic Parameters of Beta Blockers.

| Pharmacokinetic Parameter* | Log $K_m'$ | | | |
|---|---|---|---|---|
| | a | b | r | p |
| log $K_p$ | 1.38 | -0.24 | 0.88 | $<0.01 (n=8)$ |
| log $r_A$ | 1.07 | -2.01 | 0.94 | $<0.05 (n=5)$ |
| log $r_G$ | 0.96 | -1.57 | 0.47 | - |
| log $K_{BC}$ | 0.24 | -0.05 | 0.48 | - |
| log Vuss | 0.66 | 1.66 | 0.89 | $<0.01 (n=7)$ |
| log $r_T$ | 0.64 | 0.04 | 0.85 | $<0.05 (n=7)$ |
| log r | 1.60 | -1.38 | 0.88 | $<0.01 (n=8)$ |
| | Log $K_m$ | | | |
| log $K_p$ | 1.23 | -2.66 | 0.80 | $<0.05 (n=8)$ |
| log $r_A$ | 0.55 | -2.36 | 0.73 | - |
| log $r_G$ | 0.37 | -1.43 | 0.27 | - |
| log $K_{BC}$ | 0.42 | -1.06 | 0.66 | - |
| log Vuss | 0.62 | 0.40 | 0.86 | $<0.05 (n=7)$ |
| log $r_T$ | 0.68 | -1.45 | 0.93 | $<0.01 (n=7)$ |
| log r | 1.66 | -4.96 | 0.94 | $<0.01 (n=8)$ |

* See Table 14

Table 21

Linear Regression Parameters Derived from Correlations of log $K'_m$ and log $K_m$ (DMPC:CHOL 1:1 mole ratio Liposomes) and Pharmacokinetic Parameters of Beta Blockers.

| Pharmacokinetic Parameter* | a | b | r | p |
|---|---|---|---|---|
| | | Log $K'_m$ | | |
| log $K_p$ | 1.33 | -0.04 | 0.67 | - |
| log $r_A$ | 0.34 | -0.84 | 0.40 | - |
| log $r_G$ | 0.16 | -0.34 | 0.11 | - |
| log $K_{BC}$ | 0.28 | -0.003 | 0.35 | - |
| log Vuss | 0.70 | -1.70 | 0.72 | - |
| log $r_T$ | 0.80 | -0.04 | 0.82 | <0.05(n=6) |
| log r | 1.80 | -1.38 | 0.84 | <0.05(n=7) |
| | | Log $K_m$ | | |
| log $K_p$ | 0.59 | -0.49 | 0.41 | - |
| log $r_A$ | 0.15 | -0.92 | 0.31 | - |
| log $r_G$ | 0.03 | -0.25 | 0.04 | - |
| log $K_{BC}$ | 0.16 | -0.20 | 0.27 | - |
| log Vuss | 0.36 | 1.31 | 0.50 | - |
| log $r_T$ | 0.47 | -0.68 | 0.68 | - |
| log r | 1.08 | -2.88 | 0.70 | - |

* See Table 14

Table 22

Linear Regression Parameters Derived from Correlations of log $K'_m$ and log $K_m$ (DMPC:DCP 7:1 mole ratio) Liposomes and Pharmacokinetic Parameters of Beta Blockers.

| | Log $K'_m$ | | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter* | a | b | r | p |
| log $K_p$ | 0.89 | -0.18 | 0.69 | - |
| log $r_A$ | 0.73 | -1.94 | 0.96 | $<0.01 (n=5)$ |
| log $r_G$ | 0.79 | -1.78 | 0.58 | - |
| log $K_{BC}$ | 0.28 | -0.22 | 0.48 | - |
| log Vuss | 0.48 | 1.60 | 0.78 | $<0.05 (n=7)$ |
| log $r_T$ | 0.45 | 0.01 | 0.73 | - |
| log r | 1.13 | -1.48 | 0.76 | $<0.05 (n=8)$ |
| | Log $K_m$ | | | |
| log $K_p$ | 0.75 | -1.52 | 0.60 | - |
| log $r_A$ | 0.40 | -2.08 | 0.75 | - |
| log $r_G$ | 0.36 | -1.61 | 0.37 | - |
| log $K_{BC}$ | 0.25 | -0.68 | 0.46 | - |
| log Vuss | 0.43 | 0.77 | 0.73 | - |
| log $r_T$ | 0.46 | -0.98 | 0.76 | $<0.05 (n=7)$ |
| log r | 1.13 | -3.87 | 0.79 | $<0.05 (n=8)$ |

* See Table 14

Table 23

Linear Regression Parameters Derived from Correlations of log $K'_m$ and log $K_m$ (DMPC:CHOL:DCP 7:2:1 mole ratio) Liposomes and Pharmacokinetic Parameters of Beta Blockers.

| | Log $K'_m$ | | | |
|---|---|---|---|---|
| Pharmacokinetic Function* | a | b | r | p |
| log $K_p$ | 0.86 | 0.07 | 0.84 | <0.01(n=8) |
| log $r_A$ | 0.45 | -1.26 | 0.98 | <0.01(n=5) |
| log $r_G$ | 0.51 | -1.09 | 0.62 | - |
| log $K_{BC}$ | 0.33 | -0.20 | 0.79 | <0.05(n=7) |
| log Vuss | 0.45 | 1.75 | 0.94 | <0.01(n=7) |
| log $r_T$ | 0.45 | 0.11 | 0.92 | <0.01(n=7) |
| log r | 1.02 | -1.07 | 0.88 | <0.01(n=8) |
| | Log $K_m$ | | | |
| log $K_p$ | 0.73 | -1.30 | 0.75 | <0.05(n=8) |
| log $r_A$ | 0.34 | -1.74 | 0.88 | <0.05(n=5) |
| log $r_G$ | 0.34 | -1.49 | 0.50 | - |
| log $K_{BC}$ | 0.30 | -0.78 | 0.76 | <0.05(n=7) |
| log Vuss | 0.40 | 0.96 | 0.88 | <0.01(n=7) |
| log $r_T$ | 0.43 | -0.78 | 0.92 | 0.01(n=7) |
| log r | 0.98 | -3.06 | 0.87 | <0.01(n=8) |

* See Table 14

The corresponding results using log $K_m$ DMPC-4/7 ; DPPC-4/7; DMPC:CHOL- 0/7; DMPC:DCP-2/7 and DMPC:CHOL:DCP-6/7. Among all the liposome compositions, DMPC:CHOL:DCP (7:2:1) yielded the best correlations (Table 23).

## I. ANALYSES OF NITROIMIDAZOLES

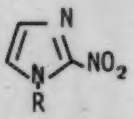
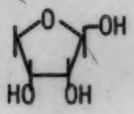
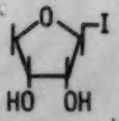
The wavelengths of maximum absorbance $\lambda$max, of nitroimidazole in saline solution were scanned in a Beckman spectrophotometer in the UV region (200-360 nm) to obtain $\lambda$max. Their chemical structures and molecular weights are shown in Table 24. Linear calibration curves which obeyed Beers law over the concentration range of 4.4-72.0 mcg/ml were obtained for the nitroimidazoles as shown in Figures 27-29. Regression analysis of the experimental points yielded slopes ranging from 0.018 to 0.042, intercepts from -0.030 to 0.017 and correlation coefficients, r, from 0.999 to 1.000.

## J. PARTITIONING OF NITROIMIDAZOLES

Nitroimidazoles are polar and highly water-soluble compounds. Consequently low partition coefficients were obtained in the n-octanol-saline system. Log K and log $K_m$ values in the n-octanol-saline system and liposomes of various compositions, respectively are provided in Table 25 for the nitroimidazoles. Nitroimidazoles are unionized at neutral pH, therefore partition coefficients measured were

## Table 24

### Chemical Structures of Nitroimidazoles.

| Nitroimidazole | Structure | Mol.Wt. |
|---|---|---|
| |  | |
| RO-07-0741 | R=$CH_2CHOHCH_2F$ | 189 |
| RO-07-2044 | $CH_2CHOHCH_2OCH_2CF_3$ | 269 |
| SR-2508 | $CH_2CONHCH_2CH_2OH$ | 214 |
| SR-2555 | $CH_2CON(CH_2CH_2OH)_2$ | 258 |
| Misonidazole | $CH_2CHOHCH_2OCH_3$ | 201 |
| Desmethyl misonidazole | $CH_2CHOHCH_2OH$ | 187 |
| Azomycin riboside |  | 245 |
| Azomycin | H | 113 |
| Iodoazomycin riboside |  | 359 |

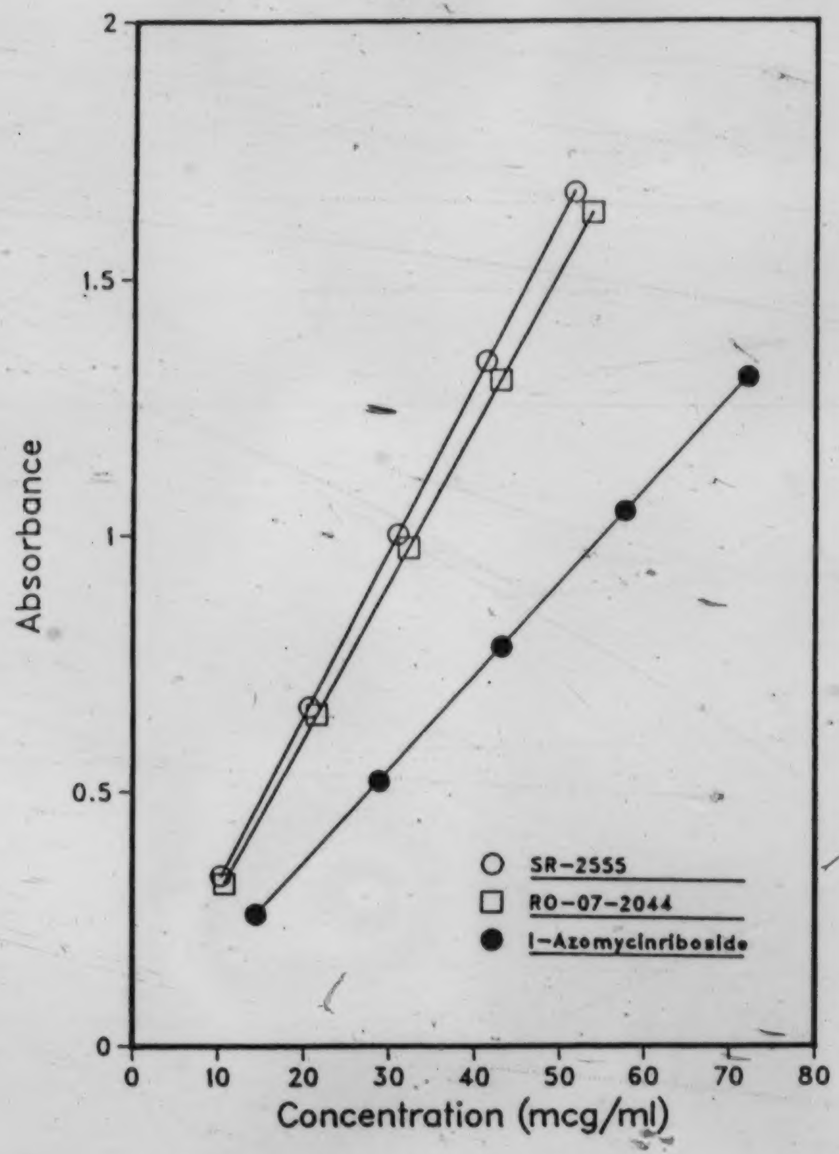


Figure 27. Beers plot of SR-2555, RO-07-2044 and I-Azomycinriboside in saline solution.

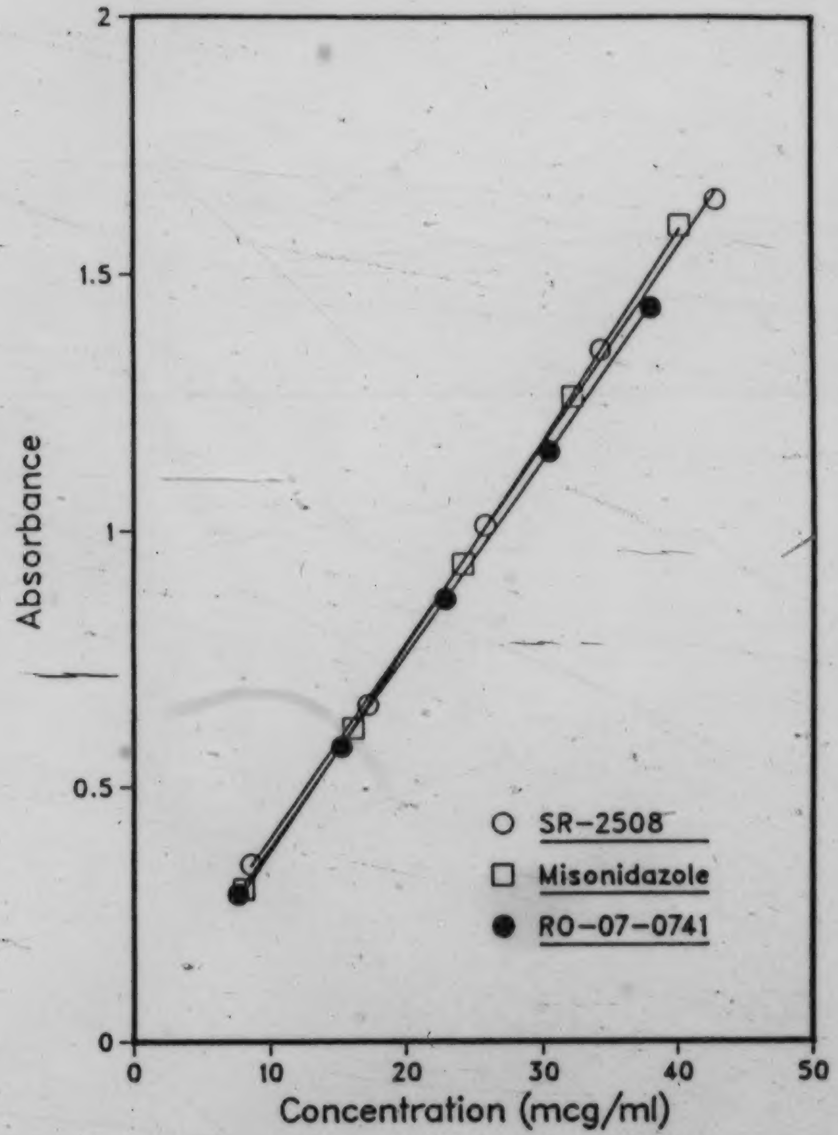


Figure 28. Beers plot of SR-2508, Misonidazole and RO-07-0741 in saline solution.

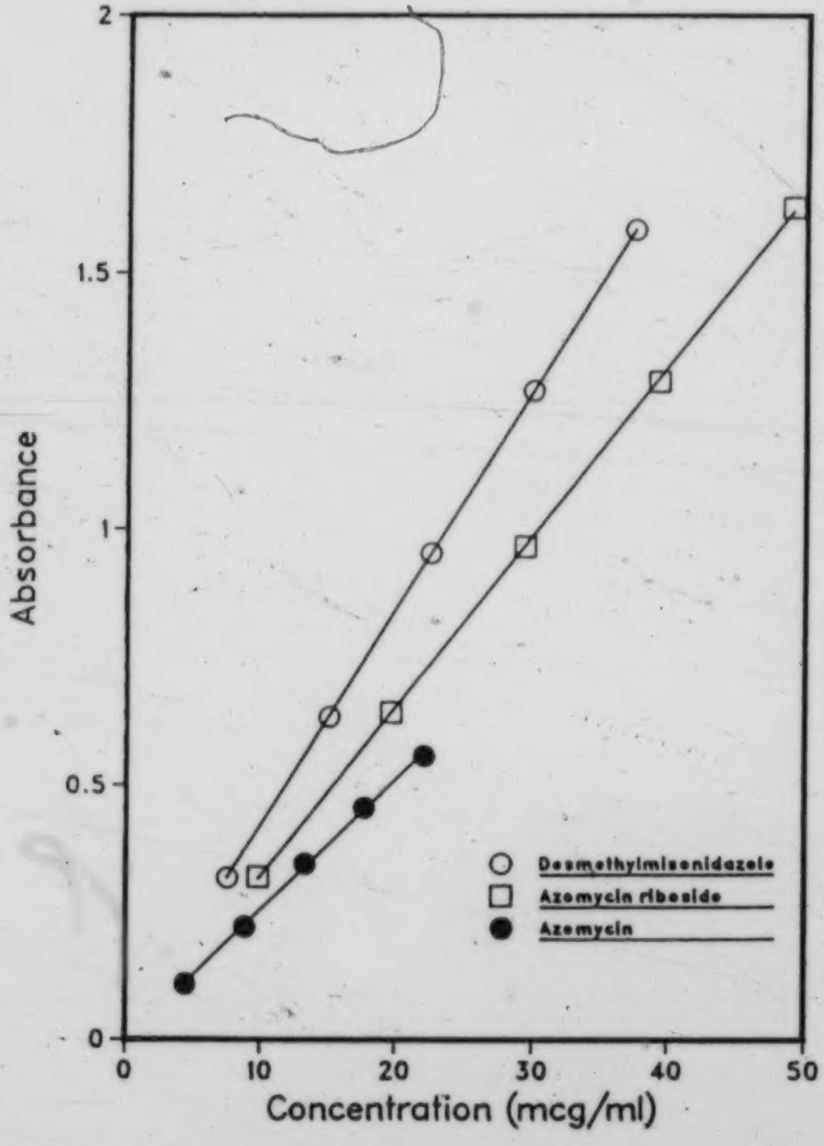


Figure 29. Beers plot of Desmethylmisonidazole, Azomycin riboside and Azomycin in saline solution.

true (K or $K_m$) values. It can be seen that in liposomes of all compositions $K_m$ was higher than in the n-octanol-saline system. In liposomes of DPPC which have longer acyl chains than DMPC $K_m$ values were decreased. At 30° C DPPC bilayers exist in the gel-state and may pose an additional structural barrier to partitioning. Reduction in $K_m$ was also the result of CHOL or DCP addition to DMPC liposomes.

## K. QSAR OF NITROIMIDAZOLES

### Correlation of Partitioning in the n-Octanol-Saline System and Liposomes

Correlations of partition coefficients of 9 nitroimidazoles in the n-octanol-saline and liposomes of various compositions are depicted in Figures 30-33 and their associated linear regression parameters are given in Table 26. The correlation coefficients, r, ranged from 0.78 to 0.92. Liposomes of DMPC:CHOL:DCP gave significant correlation at $p<0.05$ while other liposome compositions gave significant correlations at $p<0.01$. The best correlation was found with DMPC liposomes (r=0.92) (Figure 30 and Table 26).

### Correlation Between the n-Octanol-Saline Partition Coefficents and Pharmacokinetic Parameters

Pharmacokinetic parameters of nitroimidazoles (176, 177) are listed in Table 27. The linear regression

Table 25

Log K and log $K_m$ of Nitroimidazoles in Various Liposome Compositions at 30°C[a]

| Nitroimidazole | Log K | Log $K_m$ | | | |
|---|---|---|---|---|---|
| | n-Octanol-Saline System | Liposome Compositions* | | | |
| | | I | II | III | IV |
| SR-2555 | -1.64 | 0.50 | 0.08 | -0.32 | -0.23 |
| Azomycin riboside | -1.34 | 0.86 | 0.65 | 0.29 | 0.25 |
| SR-2508 | -1.34 | 0.66 | 0.37 | 0.16 | 0.14 |
| Desmethyl misonidazole | -0.85 | 0.69 | 0.49 | 0.17 | 0.21 |
| RO-07-0741 | -0.52 | 0.80 | 0.60 | 0.42 | 0.10 |
| Misonidazole | -0.37 | 0.92 | 0.74 | 0.12 | 0.14 |
| Azomycin | 0.16 | 1.13 | 0.83 | 0.54 | 0.40 |
| Iodoazomycin riboside | 0.32 | 1.29 | 0.92 | 0.44 | 0.34 |
| RO-07-2044 | 0.46 | 1.47 | 1.06 | 0.62 | 0.50 |

[a] The maximum R. S. D. was ±10%. In most cases the R. S. D. was < ±4.4%.

*I - DMPC
II - DPPC
III - DMPC:CHOL(1:1 mole ratio)
IV - DMPC:CHOL:DCP(7:2:1 mole ratio)

Table 26

Linear Regression Parameters Derived from Correlations of log K and log $K_m$ of Nitroimidazoles in Liposomes of Various Compositions.

| log $K_m$ | log K | | | |
|---|---|---|---|---|
| Liposome Composition* | a | b | r | p |
| DMPC | 0.37 | 1.14 | 0.92 | $<0.01$ |
| DPPC | 0.35 | 0.84 | 0.90 | $<0.01$ |
| DMPC:CHOL(1:1) | 0.29 | 0.44 | 0.80 | $<0.01$ |
| DMPC:CHOL:DCP(7:2:1) | 0.21 | 0.33 | 0.78 | $<0.05$ |

* mole ratios in brackets

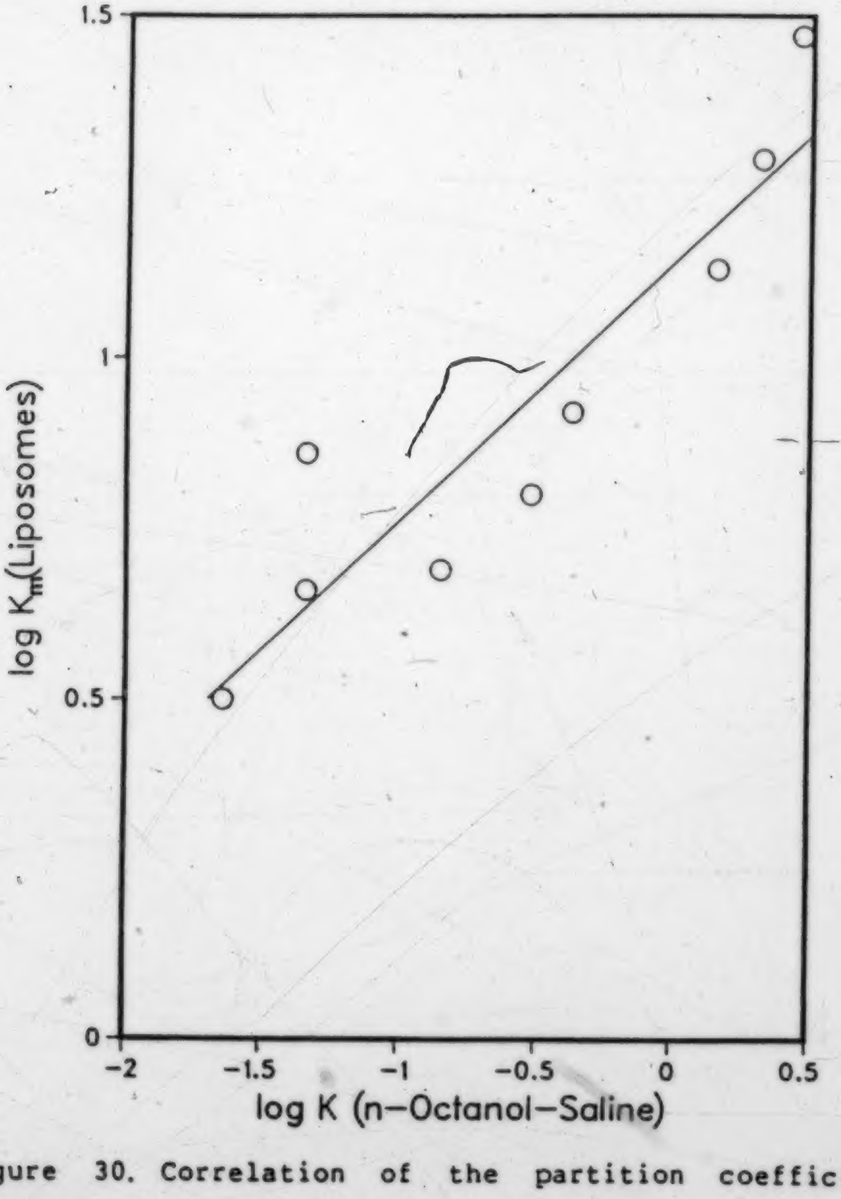


Figure 30. Correlation of the partition coefficients of nitroimidazoles in the n-octanol-saline system and DMPC liposomes at 30° C, (r=0.92).

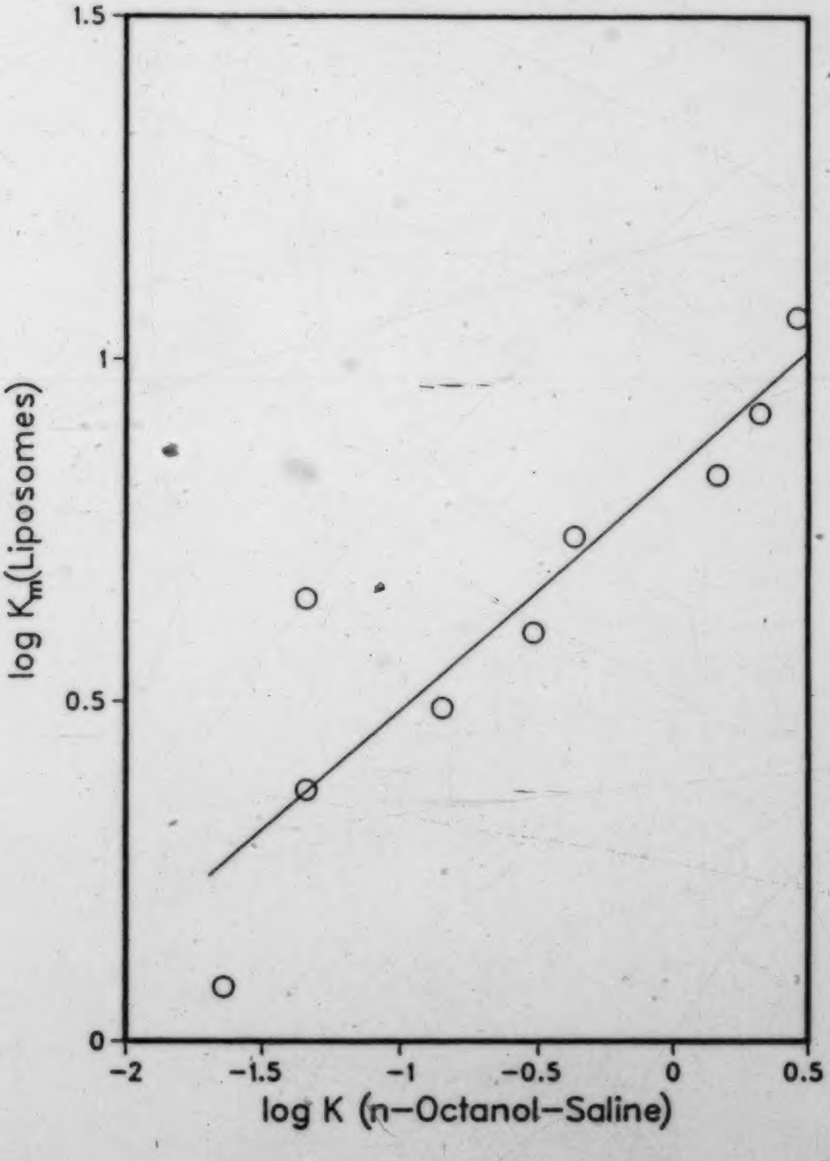


Figure 31. Correlation of the partition coefficients of nitroimidazoles in the n-octanol-saline system and DPPC liposomes at 30° C, (r=0.90).

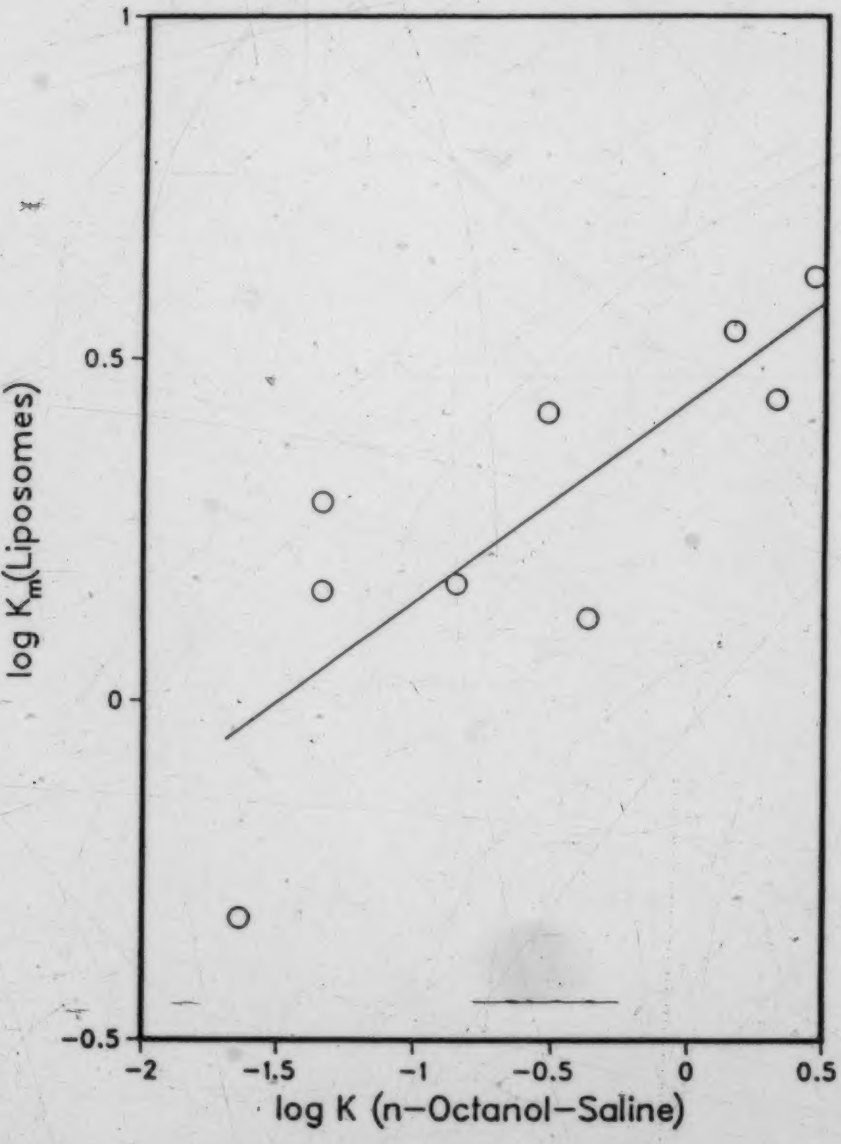


Figure 32. Correlation of the partition coefficients of nitroimidazoles in the n-octanol-saline system and DMPC:CHOL(1:1 mole ratio) liposomes at 30° C, (r=0.80).

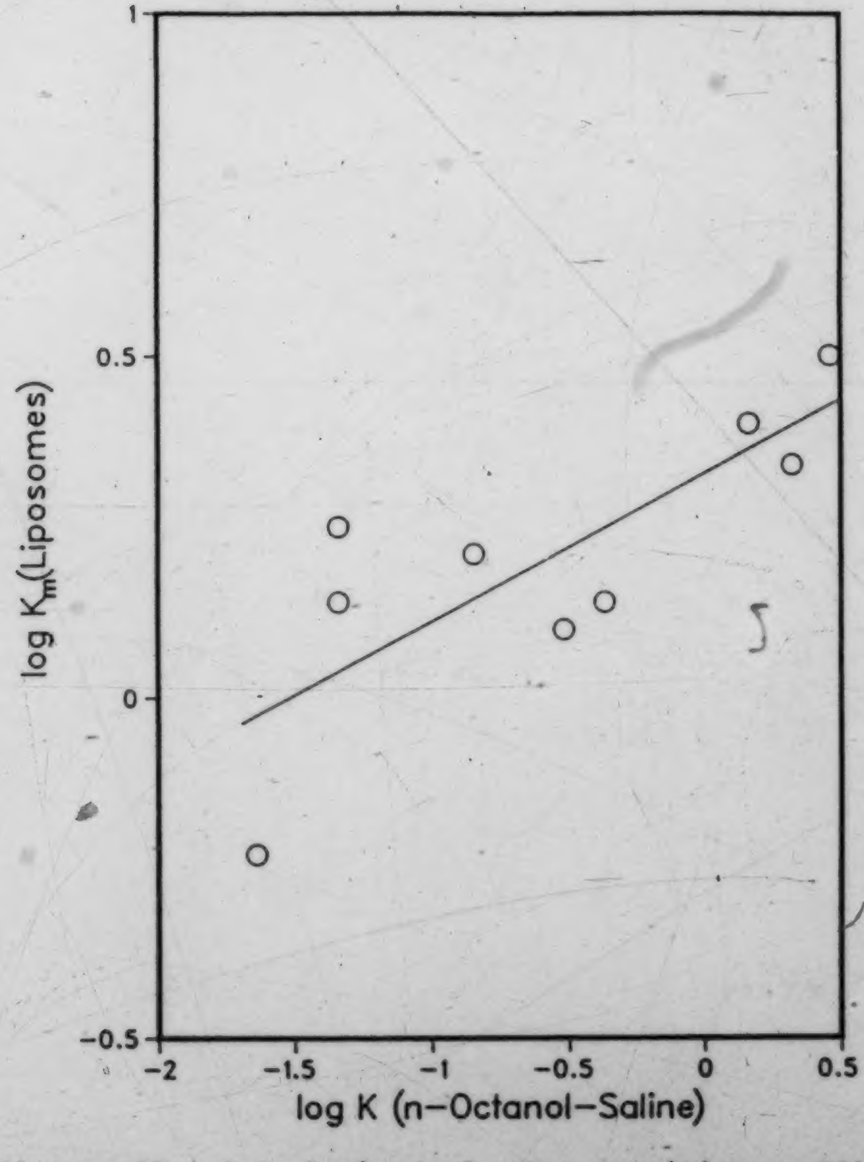


Figure 33. Correlation of the partition coefficients of nitroimidazoles in the n-octanol-saline system and DMPC:CHOL:DCP(7:2:1 mole ratio) liposomes at 30° C, (r=0.78).

parameters derived from pharmacokinetic parameters are presented in Table 28. Three pharmacokinetic parameters of 5 correlated significantly with log K. The best correlation was found using peak plasma concentrations of the nitroimidazoles (r=-0.99) whereas correlations obtained with amount of unchanged drug in urine and % oral bioavailability (r=-0.93 and 0.68 respectively) were determined to be not statistically significant.

## Correlation Between Partition Cofficients in Liposomes of Various Compositions and their Pharmacokinetic Parameters

Partition coefficients of nitroimidazoles in liposomes of DMPC, DPPC, DMPC:CHOL or DMPC:CHOL:DCP were correlated with pharmacokinetic parameters in the dog (Table 27) and the derived linear regression parameters are listed in Tables 29-32. Liposomes of DMPC:CHOL and DMPC:CHOL:DCP did not yield significant correlations with any pharmacokinetic parameters. The number of pharmacokinetic parameters that gave significant correlations with liposomes were as follows: DMPC - 3/5 (r=-0.96 to -1.00); and DPPC - 2/5 (r=-0.97 and -1.00). Oral bioavailability did not yield significant correlation with either n-octanol-saline or liposomes of any composition.

Table 27

Pharmacokinetic Parameters of Nitroimidazoles*.

| | Pharmacokinetic Parameter | | | | |
|---|---|---|---|---|---|
| Nitroimidazole | PP | PCR | UDU | OB | ALD |
| SR 2555 | 1.99 | 0.26 | 100 | 19 | 34.5 |
| SR 2508 | 1.82 | 0.21 | 88.8 | 86 | 22.9 |
| Desmethyl misonidazole | 1.06 | 0.19 | 74.7 | 57 | 18.2 |
| Misonidazole | 0.64 | 0.09 | 6.2 | 92 | 8.5 |

* Ref. 176 , 177.

PP - Peak plasma concentration ( μmole/ml)
PCR - Plasma clearance rate (liter/kg.hr)
UDU - Unchanged drug in urine (%)
OB - Oral Bioavailability (%)
ALD - Acute $LD_{50}$ (mmole/kg)

Table 28

Linear Regression Parameters Derived from Correlations of log K and Pharmacokinetic Parameters of Nitroimidazoles (n=4).

| Pharmacokinetic Parameter* | Log K<br>a | b | r | p |
|---|---|---|---|---|
| PP | -1.13 | 0.19 | -0.99 | <0.01 |
| PCR | -0.12 | 0.06 | -0.96 | <0.05 |
| UDU | -70.19 | -6.27 | -0.93 | - |
| OB | 40.46 | 105.98 | 0.68 | - |
| ALD | -18.81 | 1.28 | -0.97 | <0.05 |

* See Table 27

# Table 29

Linear Regression Parameters Derived from Correlations of log $K_m$ (DMPC Liposomes) and Pharmacokinetic Parameters of Nitroimidazoles (n=4).

| Pharmacokinetic Parameter* | Log K | | | |
|---|---|---|---|---|
| | a | b | r | p |
| PP | 3.33 | 3.68 | -0.91 | - |
| PCR | -0.41 | 0.47 | -1.00 | <0.01 |
| UDU | -232.68 | 228.56 | -0.96 | <0.05 |
| OB | 159.50 | -46.95 | 0.83 | - |
| ALD | -61.17 | 63.38 | -0.98 | <0.05 |

* See Table 27

## Table 30

Linear Regression Parameters Derived from Correlations of log $K_m$ (DPPC Liposomes) and Pharmacokinetic Parameters of Nitroimidazoles (n=4).

| Pharmacokinetic Parameter* | Log K | | | |
|---|---|---|---|---|
| | a | b | r | p |
| PP | -2.17 | 2.29 | -0.93 | - |
| PCR | -0.25 | 0.20 | -0.97 | <0.05 |
| UDU | -138.54 | 125.61 | -0.90 | - |
| OB | 100.58 | 21.26 | 0.83 | - |
| ALD | -39.40 | 37.57 | -1.00 | <0.01 |

* See Table 27

## Table 31

Linear Regression Parameters Derived from Correlations of log $K_m$ (DMPC:CHOL 1:1 mole ratio Liposomes) and Pharmacokinetic Parameters of Nitroimidazoles (n=4).

| | Log $K_m$ | | | |
|---|---|---|---|---|
| Pharmacokinetic Parameter* | a | b | r | p |
| PP | -1.60 | 1.43 | -0.59 | - |
| PCR | -0.18 | 0.19 | -0.61 | - |
| UDU | -78.49 | 69.98 | -0.44 | - |
| OB | 120.63 | 59.58 | 0.85 | - |
| ALD | -35.88 | 22.19 | -0.78 | - |

* See Table 27

Table 32

Linear Regression Parameters Derived from Correlations of log $K_m$ (DMPC:CHOL:DCP 7:2:1 mole ratio Liposomes) and Pharmacokinetic Parameters of Nitroimidazoles (n=4).

| Pharmacokinetic Parameter * | Log $K_m$ | | | |
|---|---|---|---|---|
| | a | b | r | P |
| PP | -2.09 | 1.51 | -0.65 | - |
| PCR | -0.22 | 0.20 | -0.62 | - |
| UDU | -96.76 | 73.71 | -0.46 | - |
| OB | 134.20 | 54.78 | 0.80 | - |
| ALD | -43.45 | 23.85 | -0.80 | - |

* See Table 27

# V. DISCUSSION

One of the first and particularly important observations made in these studies was the lack of dependence of log K or log $K_m$ on concentrations of PPL, MPL or TPL in either the n-octanol-buffer or liposome systems (Figure 6 and 9). This is in contrast to chlorpromazine which exhibited a marked concentration dependence of the n-octanol-buffer partition coefficient (118). The latter was explained as possibly being due to transfer of ion pairs across the oil-water interface into the oil (pH 7.4) or adsorption onto liposome surfaces. Thus, it would seem to be a reasonable assumption that beta blockers do not exhibit either of these types of behavior. These data would appear to validate Eq. 25 to obtain $K_m$ from experimental $K'_m$ required in calculations of the thermodynamics of partitioning and functional group contributions to partitioning of the beta blockers.

## A. PARTITIONING OF BETA BLOCKERS IN THE n-OCTANOL-BUFFER AND LIPOSOME SYSTEMS

### Molecular Considerations

The partitioning of many solutes between an aqueous phase and an organic phase has been shown to be entropy-controlled because of the considerable influence solutes have on the structure of water molecules (20). However, this is not always the case, particularly, when the solute molecules, associate with the molecules of the organic phase either by forming new bonds with the evolution of heat or by disrupting highly organized molecular structures in the organic phase. Usually the one action causes the other and, therefore, the net effect is compensating e.g. enthalpy-entropy compensation. However, the magnitudes of these are not always equal causing the one of greater magnitude to be dominate in driving the process, i.e. partitioning. For example, lipophilic groups tend to create positive changes in phase entropies whereas hydrophilic or polar groups tend to contribute to negative changes in phase enthalpies which may sometimes increase the phase order or structuring. Whether a solute is able to spontaneously transfer from an aqueous phase to an organic phase will be determined by the algebraic sum of the net enthalpy and the net entropy changes in the system and is indicated by the sign of the free energy term $\Delta G^{o}_{W-O}$, the spontaneous transfer of a solute from an aqueous to an organic phase is indicated by a negative $\Delta G^{o}_{W-O}$. The relative magnitudes of the $\Delta G^{o}_{W-O}$ values of the beta blockers shown in Table 9 are in order of their lipophilicities. Positive $\Delta H^{o}_{W-O}$ values indicate that polar group interactions between the beta blockers and n-octanol molecules are relatively weak and outweighed by heat energy required to break bonds in the aqueous phase. Thus, positive $\Delta H^{o}_{W-O}$ and $\Delta S^{o}_{W-O}$ describe a partitioning process that is driven by entropic energy. The low degree of partitioning of some beta blockers (i.e.

too low to detect) is indicative of water-water and water-solute interactions which exceed any lipophilic tendencies that the solutes may have. Thus, at lower temperatures the partitioning of certain beta blockers in the n-octanol-buffer system could not be detected (e.g. at 10°, PPL, BPL and APL had lower values of $K'$ than expected while $K'$ values of ABL, MPL and PDL could not be determined)

It is this balance in hydrophilic and lipophilic interactions that determines the extent to which a solute molecule will distribute between the two phases. This is particularly demonstrated by Van't Hoff plots in liposome systems. The beta blocker partitioning can be organized into 3 groups: PPL, APL, BPL (I); OFL, TPL, MPL (II); ABL, ATL, PDL (III) where lipophilicities are in the order I>II>III.

It has been often substantiated that the accuracy of thermodynamic values derived from a Van't Hoff plot is dependent on the precision of the experimental data. Furthermore, it has been asserted that thermodynamic quantities obtained from a study of the temperature-dependency of solute distribution is inappropriate, particularly when the solvents studied have high mutual solubilities and there exists molecular structures in the solvents which are temperature-sensitive (198). The n-octanol-water system is subjected to these possible limitations because of the relatively high solubility of water (27 mole%) in n-octanol (20). The $\Delta H^\circ_{W\rightarrow O}$ of propranolol (Table 11) determined by solution calorimetry

(26.00 KJ/mole) was less than that determined from the Van't Hoff plot (33.2 KJ/mole) by 27.7%. This discrepency in $\Delta H^{\circ}_{W \to O}$ may be attributed to changes in the solvent properties of n-octanol-buffer as a function of temperature. However, the discrepancy may also be partly due to the fact that the solution calorimetry experiments had to be conducted at pH 5.0 instead of pH 7.4. It has been determined that lowering the pH from 7.4 to 5.0, decreased log K' by 27.5%, suggesting a possible dependency of $\Delta H^{\circ}_{W \to O}$ on the extent of ionization of propranolol. It may be concluded from these studies that the apparent discrepancy in values of $\Delta H^{\circ}_{W \to O}$ using either the Van't Hoff plot or solution calorimetry is not significant and that thermodynamic parameters determined using temperature-dependent studies are indeed valid to describe the partitioning behavior in the n-octanol-aqueous buffer system.

The partitioning of all beta blockers was higher in liposomes than in the n-octanol-buffer system. NDL did not partition in liposomes <Tc, for the same reasons that it did not transfer to n-octanol at low temperatures. In this case, the energy required to penetrate the phospholipd bilayers in a rigid gel state of lipophilic region was not available.

Negative values of $\Delta G^{o}_{W \to L}$ for all beta blockers (except NDL) <Tc and >Tc of DMPC (Table 15) indicate the spontaneity of their partitioning in liposomes. The origin of this is found in the relative magnitudes of $\Delta H^{o}_{W \to L}$ and $\Delta S^{o}_{W \to L}$ . Below the Tc, $\Delta H^{o}_{W \to L}$ and $\Delta S^{o}_{W \to L}$ of beta blockers are negative whereas >Tc they were positive. In comparison, $\Delta H^o_{W\to L}$ and $\Delta S^o_{W\to L}$ of phenols were positive <Tc and negative >Tc and positive >Tc and <Tc for phenothiazines (118). Phenols form hydrogen bonds more readily than beta blockers and chlorpromazine was found to be sufficiently surface-active to adsorb at liposome surfaces bringing into question the phase separation method of partition coefficient calculations(118).

The ratio of $\Delta H^o_{W\to O}$ in n-octanol-buffer and $\Delta H^0_{W\to L}$ in liposome of beta blockers was found to be the same as for phenothiazines (118) >Tc. In contrast, this ratio was higher for beta blockers compared to phenothiazines <Tc. These results suggest a different mechanism of interaction of beta blockers with model membranes than the phenothiazines which have considerable surface activity (178, 179). In a membrane existing in a fluid state, such as occurs >Tc of DMPC, interaction is mainly by hydrophobically controlled partitioning (i.e. entropy dominated). On the other hand, in a membrane of a more rigid structure (<Tc) interaction through polar group association on the membrane surfaces is predominant for the beta blockers (which have low surface activity) and partitioning is enthalpy-driven.

Partition coefficient values of beta blockers in liposomes depends on the phospholipid composition. When the acyl chain length was increased by changing DMPC to DPPC the $K'_m$ values (Table 12) were decreased. When CHOL was incorporated into DMPC the $K'_m$ values were decreased compared to DMPC liposomes. Increasing the acyl chain length of a phospholipid (DPPC) or increasing the content of CHOL (Table 6) in DMPC liposomes increase the degree of order in the bilayers and yield lower values of the $K'_m$ for beta blockers. Below the Tc, CHOL hinders the phospholipid acyl chains in achieving the quasi-crystalline order that characterizes the gel-state and >Tc CHOL interferes with the lateral motions of the acyl chains that characterize the liquid-crystalline state (180). CHOL increases the permeability <Tc and decreases it >Tc. High levels of CHOL may thus tend to put the acyl chain region of the membrane into a state that is intermediate in some respects between the gel and liquid-crystalline states. The introduction of a negatively-charged binding sites in the liposome, for example DCP, increased the $K'_m$ values of beta blockers. This is due to electrostatic interaction between cationic beta blockers and the negatively-charged liposomes. A similar result was obtained for the local anesthetic, dibucaine (positively-charged), with negatively-charged (PS) liposomes (181). Charge-charge interactions must therefore play an important role in partitioning of beta blockers.

## Functional Group Contributions

As previously stated, the hydrophilic and lipophilic tendencies of solute molecules is determined by the nature of the functional groups which comprise a particular molecular structure. The behavior of a group of chemical compounds in a series in a partitioning system is firstly determined by the parent molecular structure, and secondly, by the nature and position of added functional groups. The popular approach at quantifying the contribution of a functional group to the partition coefficient of a compound, the hydrophobic substituent constant ( $\pi$ ), applied to the series of beta blockers examined in this study (Table 8), enabled an estimation of the discriminating ability of the n-octanol-buffer model partitioning system and the liposome system. Also, the more polar the functional group, the smaller was $\pi$ in either partitioning system. It was particularly noted that after polar group addition the differences in $\pi$ were greater in liposomes than in the n-octanol-buffer system indicating a greater sensitivity and selectivity of liposomes as a model membrane for evaluating solute transfer across biological membranes.

## B. INTERACTION OF PROPRANOLOL WITH MODEL AND BIOLOGICAL MEMBRANES

The interaction of PPL with membranes or membrane components has previously been reported employing erythrocytes (166) liposomes (167, 171, 172, 182) and monolayers (183). These independent studies provide strong evidence that cationic PPL undergoes specific electrostatic binding to negatively-charged sites located either outside or inside the membrane. There is also evidence that significant hydrophobic effects occur within the phospholipid bilayer to result in a major structural reorganization of the membrane. When this happens, a number of events take place. For instance, the binding of PPL to negatively-charged phospholipids may produce a domino effect by altering the protein-lipid interaction which, in turn, alters the assymetric distribution of certain phospholipids in the membrane, which in turn, alters the position and arrangements of other molecules in the membrane, etc. Likewise, binding to a basic group in a protein may also initiate a series of reactions. Tamura et al. (184) reported that treatment of human erythrocytes with cationic amphiphilic drugs increased the degradation of PC and PE by phospholipase $A_2$.

The uptake of PPL in erythrocytes, ghosts, and neutral liposomes expressed in terms of an equivalent neutral phospholipid content, supports the contention that specific binding sites for PPL exist in erythrocytes. These would appear to be distributed between protein and negatively-charged phospholipids, with the greatest affinity found towards the phospholipids (Figure 10). Lowering of the Tc of single phospholipids by local anesthetics and PPL have been reported at concentrations of 1 mM and higher (171, 182). This signifies a state of greater disorder within the membrane due to the interaction with the drug. However, in the erythrocyte membrane, local restrictions by proteins and other lipid components likely offset any temperature effects on the physical ordering states of individual phospholipids.

Thus, over the concentration range used in this study, uptake of PPL by erythrocytes was independent of temperature (25°-37° C) from 0.1 to about 1 mM and varied only slightly from 1 mM to 2.3 mM of added PPL (Figure 11). In contrast, the temperature-dependence and Van't Hoff plot of $K_m$ of PPL in liposomes clearly identified the Tc of DMPC, and it was also possible to distinguish a phase change in erythrocyte ghost membranes (Figure 14). The temperature-dependence of log $K_m$ displays a trend >Tc which is opposite to that displayed <Tc in both erythrocyte ghosts and liposome systems. The trends are also opposite between erthrocyte ghosts and liposomes <Tc or >Tc of each. At an aqueous concentration of 0.2 mM, PPL does not significantly lower the Tc of DMPC. With these points in mind, the generated thermodynamic parameters indicate a greater interaction of PPL with the erythrocyte ghost membrane than with the liposome but with a lower degree of disruption of its organizational structure (167).

The uptake of PPL increases (Figure 10), even though $K_m$ remains constant (Figure 13) with an increase in PPL concentration. A correlation between the relative hemolysis and PPL uptake in each of the membranes (Figure 34) is also apparent. These curves were constructed by determining the uptake (Figure 10) and relative hemolysis (Figure 15) at various initial PPL concentrations. Thus, the interaction of PPL with erythrocytes or ghosts produces a similar response in terms of the organizational structure which affords some

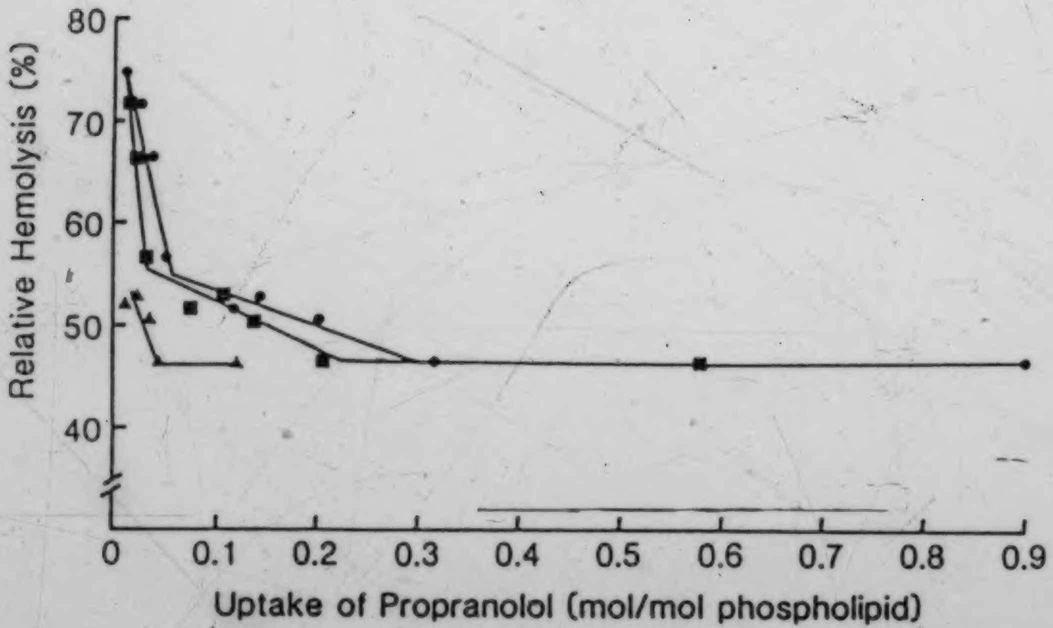


Figure 34. Relationship between the relative hemolysis of erythrocytes by concentrations of PPL and the uptake of PPL in ● erythrocytes; ■ ghosts and ▲ neutral liposomes.

degree of protection against hypotonic osmotic hemolysis, even though much of the protein and internalized solutes of the erythrocyte have been removed to produce the ghosts. In addition, these plots indicate that the relative hemolysis change with uptake is governed by at least three organizational structures imparted to the erythrocyte and ghost membranes by PPL, but only two organizational structures in the case of liposomes.

Structurally, the membrane offers a very different interface than the bulk oil-water system. The $K_m$ of PPL does not vary widely with increase in its concentration in erythrocytes, ghosts and neutral liposomes (Figure 13), but it does vary markedly with membrane weight (Figure 12) and concentration of phospholipid (169), both of which are direct determinants of the liquid crystalline state of a membrane.

The partitioning of dissociated acids or bases in biological membranes may not even resemble that found in the oil-water system. The K' of PPL between n-octanol and phosphate buffer at pH 7.4 is 30.9. However, the $K_m$ of PPL in liposomes can vary widely depending on liposome composition (Table 7 ). The membrane stabilizing behavior of PPL on cell membrane is more likely a result of structural changes occurring within the membrane, initiated then anchored by PPL, rather than simple solubility within the membrane.

## C. INTERACTION OF BETA BLOCKERS WITH UNILAMELLAR LIPOSOMES

The choline methyl protons of DMPC liposomes gave two sharp signals each having considerably different peak heights as shown in Figure 19. The downfield peak can be assigned to the choline methyl protons of phospholipid molecules in the exterior layer and the upfield one to those in the interior layer (186). Addition of $Pr^{3+}$ to the liposomes separate the internal and external peaks of the choline methyl groups in linear fashion indicating that the difference in chemical shifts of the internal and external peaks is directly proportional to the $Pr^{3+}$ concentration. Downfield shift of the external peak is due to the pseudo contact effect of the paramagnetic $Pr^{3+}$ at phosphate sites on the extravesicular head group (187).

Addition of PPL reverses the movement of the external peak due to the liposome choline methyl group by displacing $Pr^{3+}$ from the liposome surfaces as shown in Figure 16 A linear correlation between $Pr^{3+}$ displacement and $\log K'_m$ in DMPC liposomes was obtained for nonselective beta blockers but cardioselective beta blockers did not fit this correlation (Figure 21). It appears that cardioselective and nonselective beta blockers are positioned differently within the bilayer with the result that cardioselective beta blockers displace $Pr^{3+}$ more effectively than nonselective beta blockers of the same or slightly higher hydrophobicity. Calorimetric studies have shown that PPL interacts mainly with the nonpolar part of the bilayer (188). Similar localization has been suggested for tetracaine on the basis of NMR studies (189).

It would appear that nonselective beta blockers which have ortho or meta-substitution are sterically hindered from interacting with the polar head groups of DMPC. But increased partitioning offsets this problem of steric hinderance. On the other hand, para-substitution of the cardioselective agents with low $K'_m$'s permits polar group interaction with the membrane. Thus, the displacement of polyvalent cations from liposomes may be taken as indirect evidence of the ability of a beta blocker to either penetrate the hydrophobic bilayer or to bind with the polar head groups of liposomes. These findings suggest that beta blockers which interact hydrophobically are nonselective whereas those which interact preferentially with the polar head groups of liposomes are cardioselective.

## D. QSAR OF BETA BLOCKERS

A plot of log K (n-octanol-buffer) vs log $K_m$ (DMPC liposomes) is given in Figure 22 and shows generally that the hydrophobicities of the beta blockers are correlated in the n-octanol-buffer and liposome systems. However, ABL does not correlate as well as the others. Correlations of this type have been found with other solutes (30, 31), but this does not necessarily mean that the two systems behave similarly thermodynamically (117).

Linear relationships obtained from enthalpy-entropy compensation plots suggest a single mechanism of transfer for a series of solutes or solvents (40). However, in the n-octanol-0.15 M NaCl solution system, no linear relationship was obtained between $\Delta G$ and $\Delta H$ for phenolic solutes (117). Likewise, a poor correlation between $\Delta G^o_{W\rightarrow O}$ and $\Delta H^o_{W\rightarrow O}$ was obtained for the beta blockers in the n-octanol-buffer system (r=-0.543) and in DMPC liposomes <Tc (r=-0.493). However, a positive correlation was obtained in DMPC liposomes >Tc (r=0.767) as shown in Figure 35. This is not a strong indication that all of the beta blockers interact with phospholipid bilayer molecules in exactly the same manner but it does suggest that there are no major structural alterations in the lipid environment over the temperature range of the study, whereas this may be the case in n-octanol because of the existence of structures of water-centered aggregates (20). By varying the liposome composition, correlations can be improved as given in Table 13. Although either model system is able to differentiate this series of beta blockers on the basis of their relative hydrophobicities, the thermodynamic approach shows how structural organization and rigidity of the lipid phase might account for the selectivity of molecules through the interactive behaviour of certain functional groups. Thus, ABL and NDL were found to be selected by the one system (liposomes) but not the other (n-octanol-buffer).

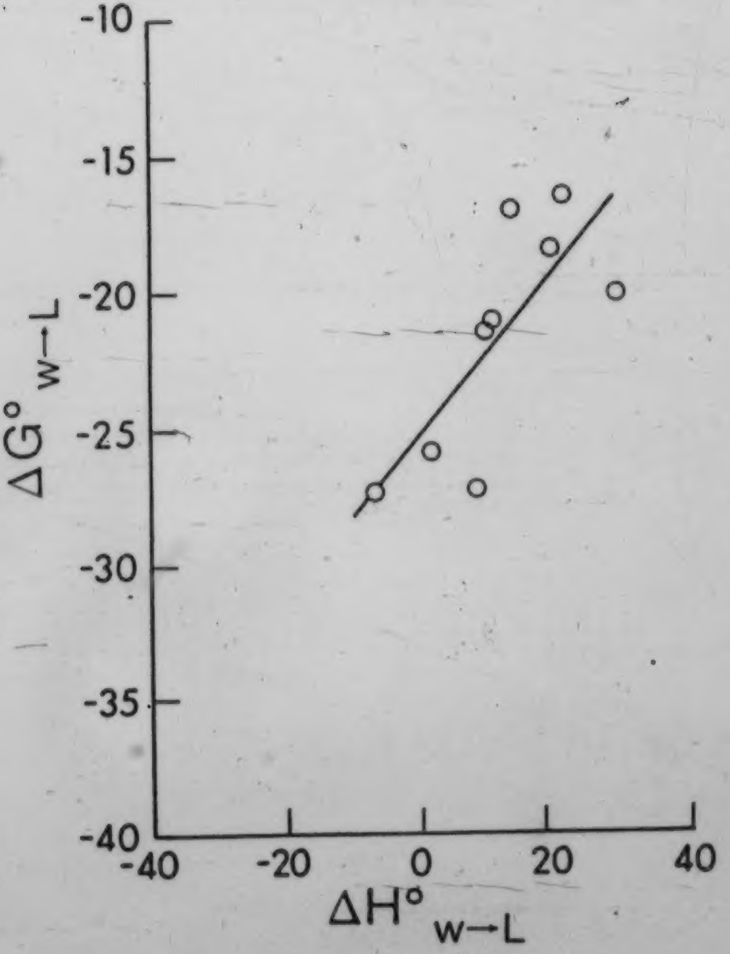


Figure 35. Enthalpy-entropy compensation of beta blockers in DMPC liposomes above the Tc (r=0.767).

When K' or $K'_m$ and K or $K_m$ values were used to correlate with pharmacokinetic parameters, the correlations were better when K' or $K'_m$ values were used in both the n-octanol-buffer and liposome systems. These results indicate that the polar group as well as hydrophobic interactions are the determinants of the pharmacokinetic parameters. This is also in accordance with the results of published studies on structure and pharmacokinetic relationships (174, 190-192).

The best correlation between partition coefficient and corneal penetration behavior was found with liposomes of DMPC:CHOL composition, whereas there was no significant correlation when n-octanol-buffer values were used. In comparison, the correlation between partition coefficients of steroids and n-alkyl p-aminophenobenzoate homologues in the n-octanol-water system and corneal penetration behavior was found to be parabolic (193, 194). Next correlation coefficients were higher using K' or $K'_m$ values, (Table 17), suggesting that hydrophilic and hydrophobic interactions are the factors in their corneal penetration. These results also indicate a limitation in the use of the n-octanol-buffer system in QSAR studies whereas the liposome system has been demonstrated as having better overall usefulness in QSAR studies. The advantage of the liposome system is that its structure can be altered in terms of charge, permeability and size to improve correlations which is not possible in an oil-water system. Correlation studies with pharmacokinetic parameters or corneal penetration behavior clearly indicate the differences in the interactions of beta blockers with different biological membranes. The charged liposome model gave the best correlation with pharmacokinetic parameters whereas liposomes having decreased fluidity and a more ordered structure better described corneal penetration behavior.

Predictions of the value of the pharmacokinetic parameters, Vuss, $r_A$, and corneal penetration behavior were made in the n-octanol-buffer and liposome systems of different phospholipid compositions (Tables 33-35). The predictions were made by using previously obtained regressions of the pharmacokinetic parameters and corneal penetration behavior with K' or $K'_m$. Using this procedure allows estimates of the biological properties of new compounds *in vivo* provided that *in vivo--in vitro* relationships were known for a sufficient number of congeners and that the *in vivo* characteristics of new compounds were within the range of the tested congeners. Rational drug design would then be possible.

Comparisons of predicted values of pharmacokinetic parameters with literature values (174) shows that DMPC, DPPC and DMPC:CHOL:DCP liposome systems are superior to the n-octanol-buffer system for predicting Vuss while DPPC and DMPC:CHOL:DCP were better in predictions of $r_A$. The least average % error was found using liposomes of DMPC:CHOL:DCP. These results indicate that the pharmacokinetic behavior of

Table 33

Comparison of Membrane - Predicted and Literature Values of Steady-State Volumes of Distribution (Vuss) of Beta Blockers.

| Beta Blocker | Predicted (Vuss,L) | | | | | | Literature Values(174) |
|---|---|---|---|---|---|---|---|
| | n-Octanol-Buffer System | Liposomes | | | | | |
| | | I | II | III | IV | V | |
| Propranolol | 864 | 1,606 | 941 | 567 | 790 | 1,271 | 1,950 |
| Alprenolol | 708 | 226 | 514 | 483 | 576 | 636 | 316 |
| Metoprolol | 156 | 226 | 316 | 540 | 276 | 202 | 240 |
| Pindolol | 133 | 295 | 326 | 109 | 227 | 186 | 200 |
| Nadolol | - | 123 | 120 | - | 104 | 156 | 186 |
| Acebutolol | 226 | 100 | 124 | 150 | 96 | 141 | 126 |
| Atenolol | 103 | 185 | 74 | 122 | 193 | 82 | 79 |
| Av. % error | 59 | 33 | 39 | 78 | 55 | 23 | |

Using regression equations from Tables 15, 18-23

I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:DCP (7:1 mole ratio)
V - DMPC:CHOL:DCP (7:2:1 mole ratio)

Table 34

Comparison of Membrane - Predicted and Literature Values of the Ratio of Fraction of Beta Blocker Bound and Unbound to Albumin ($r_A$).

| Beta Blocker | Predicted ($r_A$) | | | | | | Literature Values(174) |
|---|---|---|---|---|---|---|---|
| | n-Octanol-Buffer System | Liposomes | | | | | |
| | | I | II | III | IV | V | |
| Propranolol | 0.94 | 1.13 | 1.36 | 0.47 | 1.13 | 1.26 | 1.23 |
| Alprenolol | 0.78 | 0.26 | 0.51 | 0.44 | 0.69 | 0.63 | 0.66 |
| Oxprenolol | 0.36 | 0.36 | 0.21 | 0.41 | 0.28 | 0.28 | 0.29 |
| Pindolol | 0.16 | 0.31 | 0.24 | 0.21 | 0.17 | 0.19 | 0.22 |
| Metoprolol | 0.19 | 0.26 | 0.23 | 0.46 | 0.22 | 0.20 | 0.16 |
| Av. % error | 24 | 50 | 23 | 62 | 20 | 9 | |

Using regression equations from Tables 15, 18-23

I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:DCP (7:1 mole ratio)
V - DMPC:CHOL:DCP (7:2:1 mole ratio)

Table 35

Comparison of Membrane - Predicted and Literature Values of Corneal Penetration ($P_T$) of Beta Blockers.

| | Predicted ($P_T$) | | | | | | |
|---|---|---|---|---|---|---|---|
| Beta Blocker | n-Octanol-Buffer System | Liposomes | | | | | Literature Values(175 |
| | | I | II | III | IV | V | |
| Propranolol | 66.40 | 110.00 | 116.00 | 45.50 | 91.21 | 115.00 | 57.50 |
| Oxprenolol | 10.80 | 8.84 | 10.80 | 19.30 | 11.83 | 7.90 | 27.50 |
| Metoprolol | 3.19 | 4.29 | 11.90 | 39.80 | 8.35 | 4.39 | 24.09 |
| Nadolol | - | 1.57 | 1.57 | - | 0.91 | 2.78 | 1.02 |
| Acebulolol | 6.17 | 1.11 | 1.67 | 1.06 | 0.74 | 2.31 | 0.85 |
| Atenolol | 1.54 | 3.09 | 0.57 | 0.59 | 3.67 | 0.88 | 0.68 |
| Av. % error | 192 | 142 | 70 | 29 | 107 | 149 | |

Using regression equations from Table 17

I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:DCP (7:1 mole ratio)
V - DMPC:CHOL DCP (7:2:1 mole ratio)

a new beta blocker can be reasonably predicted when a negatively-charged liposome system is used in distribution studies.

The prediction of the corneal penetration behavior of beta blockers in rabbits was best obtained from liposomes of all compositions studied rather than the n-octanol-buffer system, and the corneal penetration behavior of a new beta blocker can be predicted best from DMPC:CHOL liposomes.

Although the relative hydrophobicities of group of compounds can be determined in the n-octanol-buffer system, a liposome system may also be used. Thermodynamically $\Delta G_{w-O}$ and $\Delta G^0_{w \rightarrow L}$ are temperature-dependent but in addition $\Delta H^0_{w \rightarrow L}$ and $\Delta S^0_{w \rightarrow L}$ change dramatically when the temperature is varied over the Tc. Comparison of uptakes of PPL in liposomes and in erythrocytes indicated that the lipid bilayer structure plays a major role. Liposomes have the flexibility of being able to modify their size, surface charge and structures (gel or fluid) which produce changes in the partitioning or uptake of solutes, their thermodynamic analysis, functional group contributions of molecules and specific drug-membrane interactions (using sensitive techniques such as ESR, NMR etc.,) which relate more closely to biological membranes. Depending on the particular group of compounds, correlations between partition coefficients and biological activities may be significantly improved by altering the liposome composition and structure. This flexibility of liposomes is not available in the octanol-water system.

## E. PARTITIONING OF NITROIMIDAZOLES IN THE n-OCTANOL-SALINE AND LIPOSOME SYSTEMS

Nitroimidazoles are hydrophilic compounds, hence, their K or $K_m$ values were quite low. Log K values in n-octanol-saline system ranged from -1.64 to 0.46. Liposomes of any phospholipid composition (Table 25) yielded higher values of $K_m$ than the n-octanol-saline system. DMPC liposomes gave the highest log $K_m$ values when measured >Tc. When DPPC and DMPC:CHOL (1:1 mole ratio) liposomes were used the log $K_m$ values were lower, similar to that of the beta blockers. When negatively-charged liposomes were used (DMPC:CHOL:DCP) the log $K_m$ values did not increase in contrast to that observed with beta blockers. Since nitroimidazoles are completely unionized in saline solution at neutral pH, there are no electrostatic interactions. The presence of CHOL increases the ordered structure of bilayers and reduces the permeability of liposomes to solutes. Therefore, lower values of log $K_m$ are observed in negatively-charged liposomes. Log K values of SR-2508 and azomycin riboside were the same in the n-octanol-saline system but the log $K_m$ values were different in liposomes of any composition. This is another example of the selectivity of liposomes as a membrane model.

## F. QSAR OF NITROIMIDAZOLES

Correlation of the partition coefficients of nitroimidazoles in the n-octanol-saline system and in liposomes of various compositions were significant at the $p<0.01$ level (Table 26). However, the best correlation found for this group of compounds was with neutral liposomes (DMPC) >Tc. These results suggest that, overall, the n-octanol-saline system and liposomes may equally predict the behavior of the nitroimidazoles with respect to their activities in so for as these are influenced by the partition coefficient. Variation of the correlation level with liposome composition suggests that various types of membrane structure and composition can offset the role of partitioning in describing drug action.

Correlations between partition coefficients and pharmacokinetic parameters were comparable in the n-octanol-saline system and in DMPC or DPPC liposomes. Liposomes of pure phospholipids correlated better than liposomes of mixed composition. DMPC:CHOL and DMPC:CHOL:DCP did not correlate significantly with any of the pharmacokinetic parameters (Tables 31, 32). Since the nitroimidazoles are completely unionized at physiological pH negatively-charged liposomes (DMPC:CHOL:DCP) had no effect on the correlation of partition coefficients with pharmacokinetic parameters (contrary to that observed with beta blockers).

Table 36

Comparison of Membrane - Predicted and Literature Values of Acute LD$_{50}$ of Nitroimidazoles.

| Nitroimidazole | Predicted LD$_{50}$(mM/Kg) | | | | | Literature Values(177) |
|---|---|---|---|---|---|---|
| | n-Octanol-Saline System | Liposomes | | | | |
| | | I | II | III | IV | |
| SR 2555 | 32.12 | 32.80 | 34.42 | 33.67 | 33.84 | 34.50 |
| SR 2508 | 26.48 | 23.01 | 22.99 | 16.45 | 17.77 | 22.90 |
| Desmethyl misonidazole | 17.26 | 21.18 | 18.27 | 16.09 | 14.72 | 18.20 |
| Misonidazole | 8.24 | 7.11 | 8.42 | 17.88 | 17.77 | 8.50 |
| Av. % error | 8 | 10 | 0.5 | 27 | 27 | |

Using regression equations from Tables 28-32

I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:CHOL:DCP (7:2:1 mole ratio)

Table 37

Comparison of Membrane - Predicted and Literature Values of Plasma Clearance Rate (PCR) of Nitroimidazoles.

| Nitro-imidazole | Predicted (PCR, Liter/kg.hr) | | | | | Literature Values(176) |
|---|---|---|---|---|---|---|
| | n-Octanol-Buffer System | Liposomes | | | | |
| | | I | II | III | IV | |
| SR 2555 | 0.26 | 0.27 | 0.27 | 0.25 | 0.25 | 0.26 |
| SR 2508 | 0.22 | 0.20 | 0.20 | 0.16 | 0.17 | 0.21 |
| Desmethyl misonidazole | 0.16 | 0.19 | 0.17 | 0.16 | 0.19 | 0.19 |
| Misonidazole | 0.10 | 0.09 | 0.10 | 0.17 | 0.17 | 0.09 |
| Av. % error | 11 | 2 | 8 | 25 | 223 | |

Using regression equations from Tables 28-32

I - DMPC
II - DPPC
III - DMPC:CHOL (1:1 mole ratio)
IV - DMPC:CHOL:DCP (7:2:1 mole ratio)

Predictions of the pharmacokinetic parameters, ALD and PCR in the n-octanol-saline and liposome systems of different phospholipid compositions are shown in Tables 36 and 37. The predictions were made by using previously obtained regressions of the pharmacokinetic parameters with $K_m$. Comparisons of predicted values of pharmacokinetic parameters and their literature values (176, 177) show that DMPC and DPPC liposome systems are superior to the n-octanol-saline system for predicting PCR while DPPC gave better predictions of ALD. The least average % errors of the predictions were found with DMPC (2%) and DPPC (0.5%) liposomes. Thus, the pharmacokinetic behavior of a new nitroimidazole can be reasonably predicted using a neutral liposome system and, also, the development of radiosensitizers which are less toxic than misonidazole may be improved by application of the partition coefficient as suggested by Brown and Workman (195).

# VI. SUMMARY AND CONCLUSIONS

Liposomes have been well established as models to mimic biological membranes since lipid bilayers are the backbone structure of biological membranes. The bulk oil-water system is often too simplified a model to describe the properties of drug-membrane interactions which are important in membrane uptake and transport of drugs. On the other hand the liposome has been shown to be a more selective distribution model to study the QSAR of beta blockers and nitroimidazoles.

Partition coefficients were measured in n-octanol-buffer and liposomes of varying compositions. Thermodynamic analyses were carried out for beta blockers in the n-octanol-buffer system and DMPC liposomes. The specific interaction of PPL with human erythrocytes, erythrocyte ghosts and liposomes has been investigated and protection against hemolysis of erythrocytes was measured. Interaction of cardioselective and nonselective beta blockers with unilamellar DMPC liposomes has been studied using NMR. QSAR were determined for beta blockers based on pharmacokinetic properties, corneal penetration and partition coefficients in the n-octanol-buffer and liposome systems. Similarly, QSAR were determined for nitroimidazoles based on pharmacokinetic properties and partition coefficients in the n-octanol-saline and liposome systems.

1. In general, the partition coefficient of beta blockers and nitroimidazoles is greater in liposomes than in the

n-octanol-buffer or n-octanol-saline system.

2. Enthalpies and entropies of partition were generally lower below the phase transition temperature of the phospholipid and in comparison to the n-octanol-buffer system.
3. Enthalpy-entropy compensation exists for beta blockers in liposomes >Tc but not <Tc or in the n-octanol-buffer system.
4. $\Delta H^{o}_{W \rightarrow O}$ values of propranolol determined by solution calorimeter were lower than values obtained from temperature-dependent studies.
5. $K_m$'s of beta blockers and nitroimidazoles were lower in DPPC and DMPC:CHOL liposomes compared to DMPC.
6. In negatively-charged liposomes of DMPC:CHOL:DCP the $K_m$'s of beta blockers but not the nitroimidazoles were higher.
7. Very low concentrations of CHOL in DMPC liposomes increased the partitioning of propranolol but partitioning decreased as the concentration of CHOL increased.
8. The uptake of propranolol was greatest in erythrocytes, slightly less in ghosts and considerably less in neutral liposomes.
9. The maximum amount of protection against osmotic hemolysis of erythrocytes by propranolol was about 54%.
10. The membrane-stabilizing behavior of propranolol on cell membranes is more likely a result of structural changes

occurring within the membrane, rather than simple solubility within the membrane.

11. Addition of $Pr^{3+}$ to the aqueous phase in NMR studies increased the splitting of the trimethylammonium group signals arising from the internal and external polar groups of the phospholipid molecules in the bilayers.
12. The splitting of the $^1$H-NMR signal from the internal and external trimethylammonium groups of DMPC due to $Pr^{3+}$ increased linearly.
13. The addition of beta blockers reversed the effect of $Pr^{3+}$.
14. $^1$H-NMR analysis of liposome-associated beta blockers enables distinction between their nonselective and cardioselective actions.
15. Statistically significant correlations were found for partitioning in the n-octanol-buffer and liposome systems. The best correlation for beta blockers was found with a liposome composition of DMPC:CHOL:DCP (7:2:1 mole ratio) whereas for nitroimidazoles DMPC yielded the better correlation.
16. Log K' of beta blockers in the n-octanol-buffer and log $K'_m$ in liposomes correlated with pharmacokinetic parameters and corneal penetration behavior better than log K or log $K_m$.
17. Partitioning of nitroimidazoles in the n-octanol-saline and liposome systems correlated with pharmacokinetic parameters best with liposomes of pure phospholipid

compositions (DMPC and DPPC).

18. Predictions of pharmacokinetic parameters of beta blockers and nitroimidazoles and the corneal penetration behavior of beta blockers from the structured liposome model agreed more closely with literature values than from those predicted from the n-octanol-buffer (or saline) system.

19. Both the n-octanol-buffer and the liposome system can be employed to measure the relative hydrophobicities of congeners of drugs in a series. The flexibility and selectivity which can be obtained with liposomes in studying partitioning behavior, and drug-membrane interactions for QSAR is not possible with the simple oil-water system.

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# APPENDIX

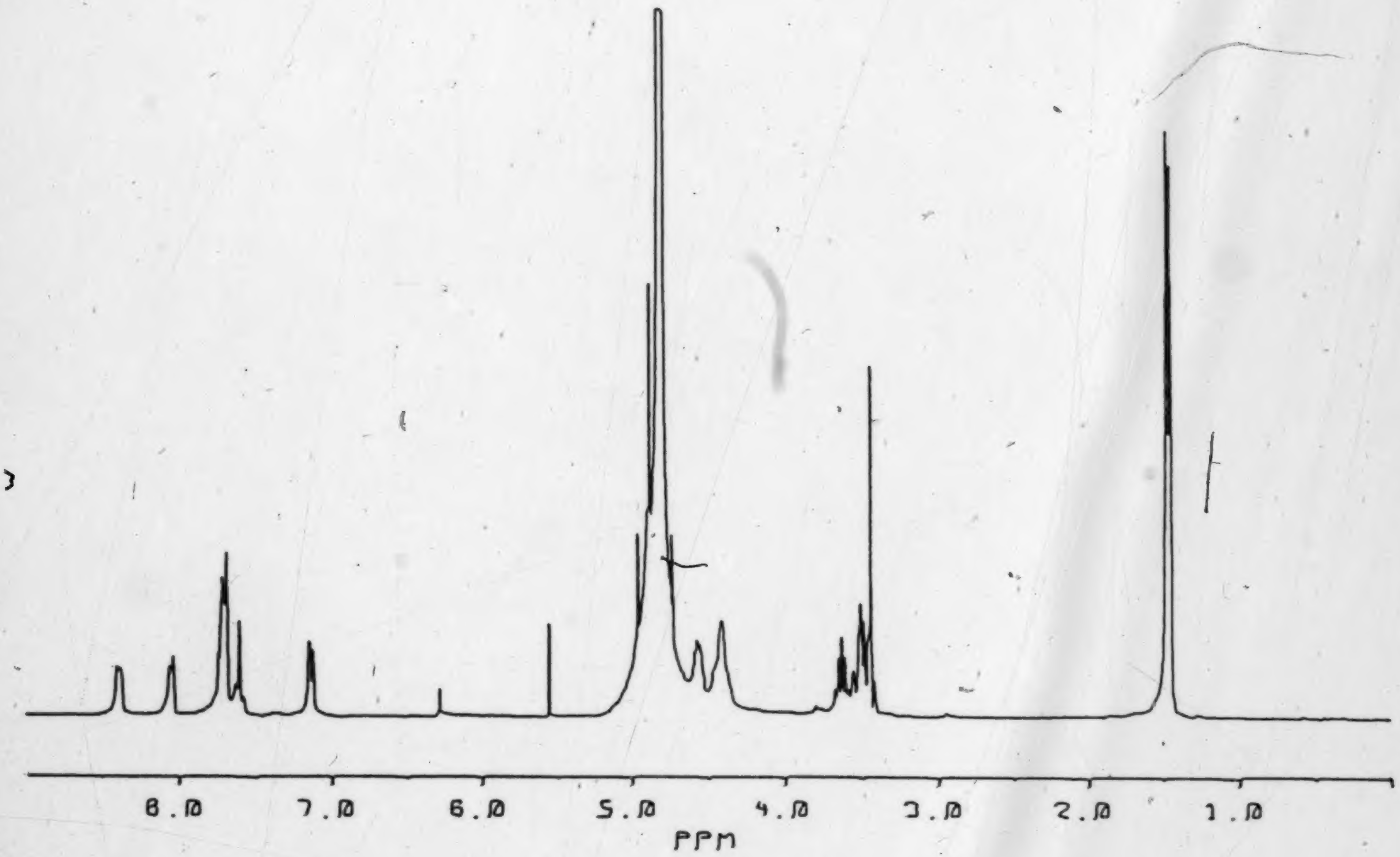

Figure A-1. $^1$H-NMR spectrum of PPL.

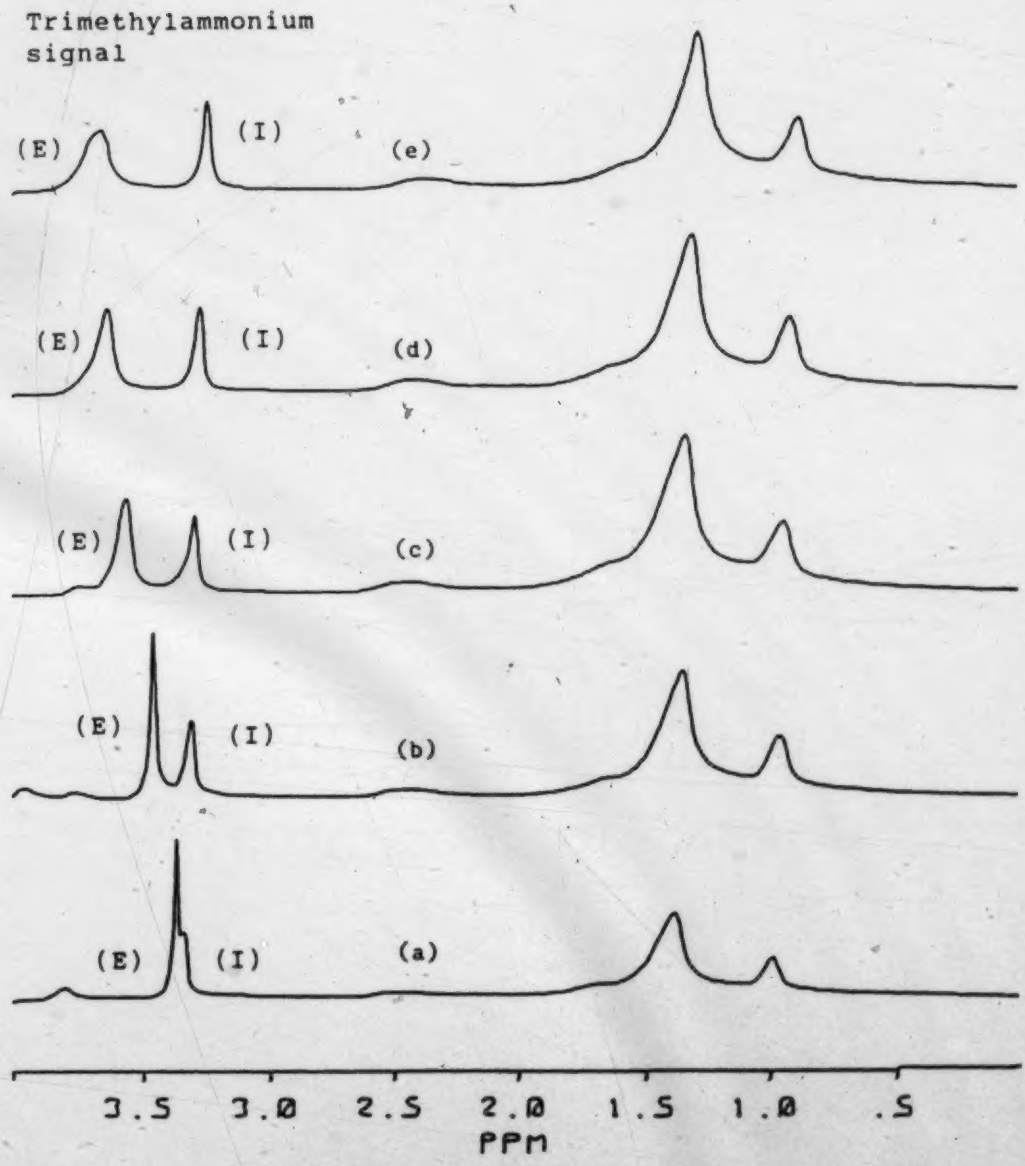

Figure A-2. $^1$H-NMR spectra of DMPC(14.4 mM) + Pr$^{3+}$ : a)0 mM; b) 1 mM; c) 4 mM; d) 8 mM and e) 10 mM.

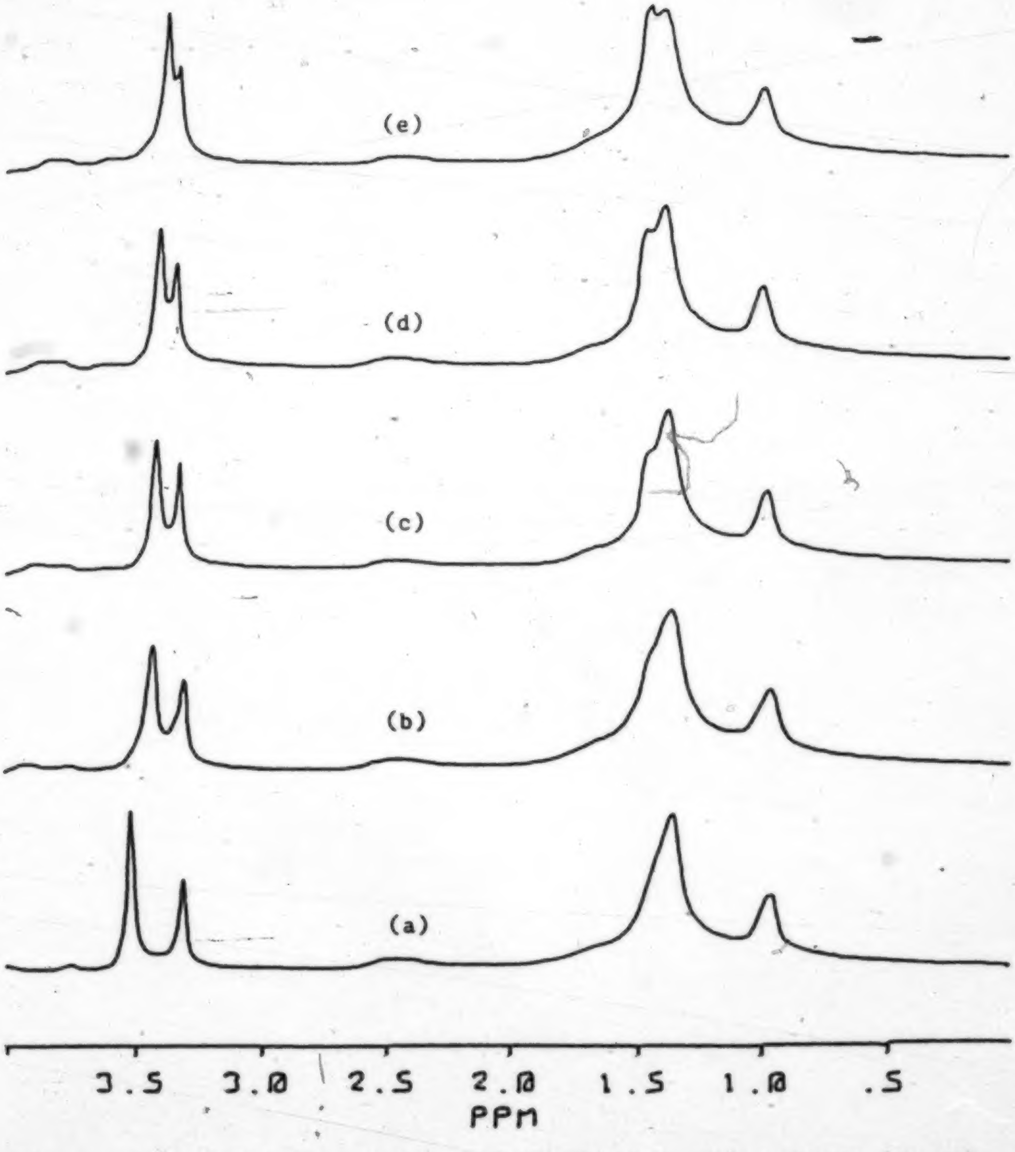

Figure A-3. $^1$H-NMR spectra of DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) + APL : a) 0 mM; b) 2 mM; c) 4 mM; d) 6 mM and e) 8 mM.

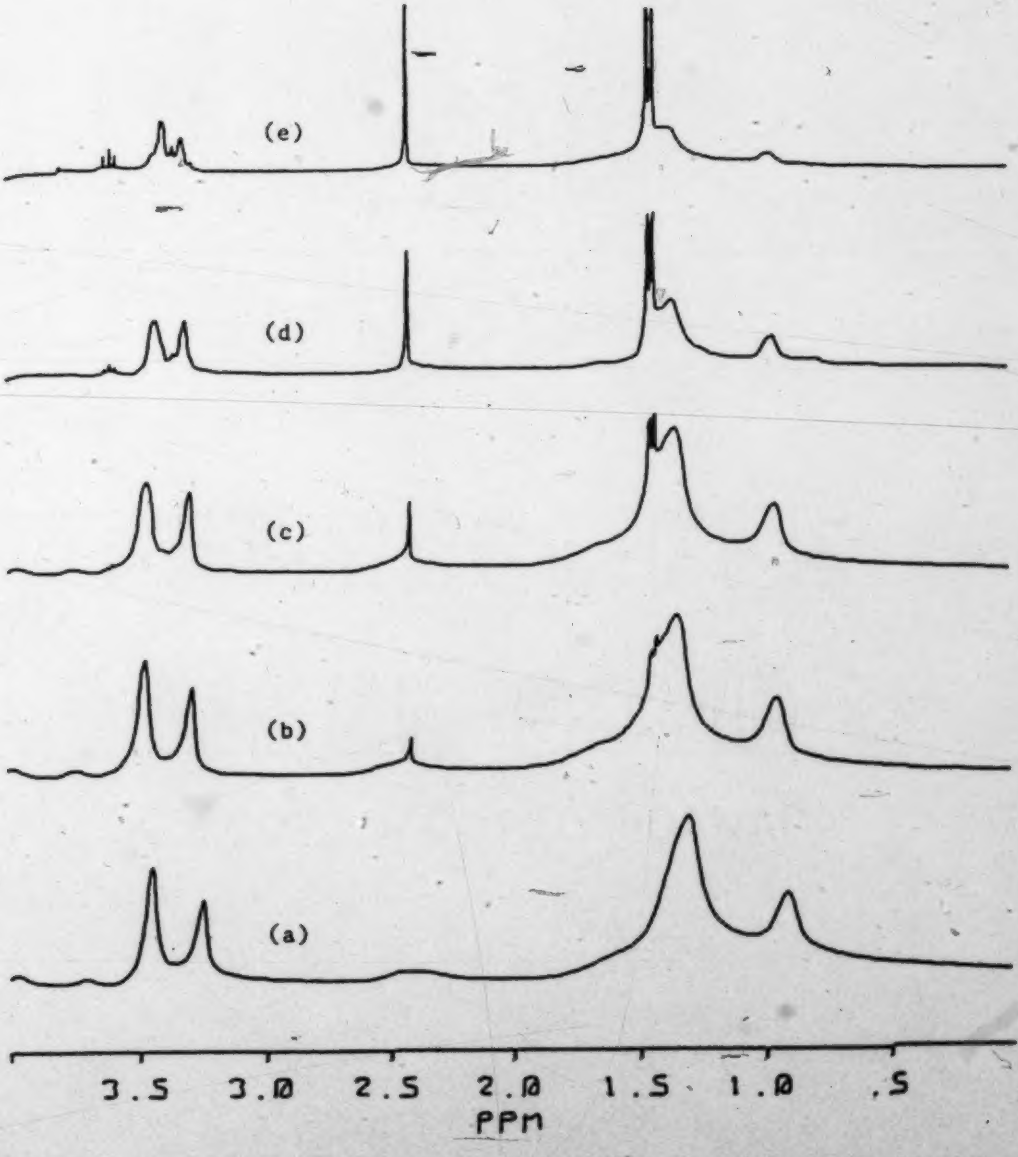

Figure A-4. 'H-NMR spectra of DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) + TPL : a) 0 mM; b) 2 mM; c) 5 mM; d) 15 mM and e) 25 mM.

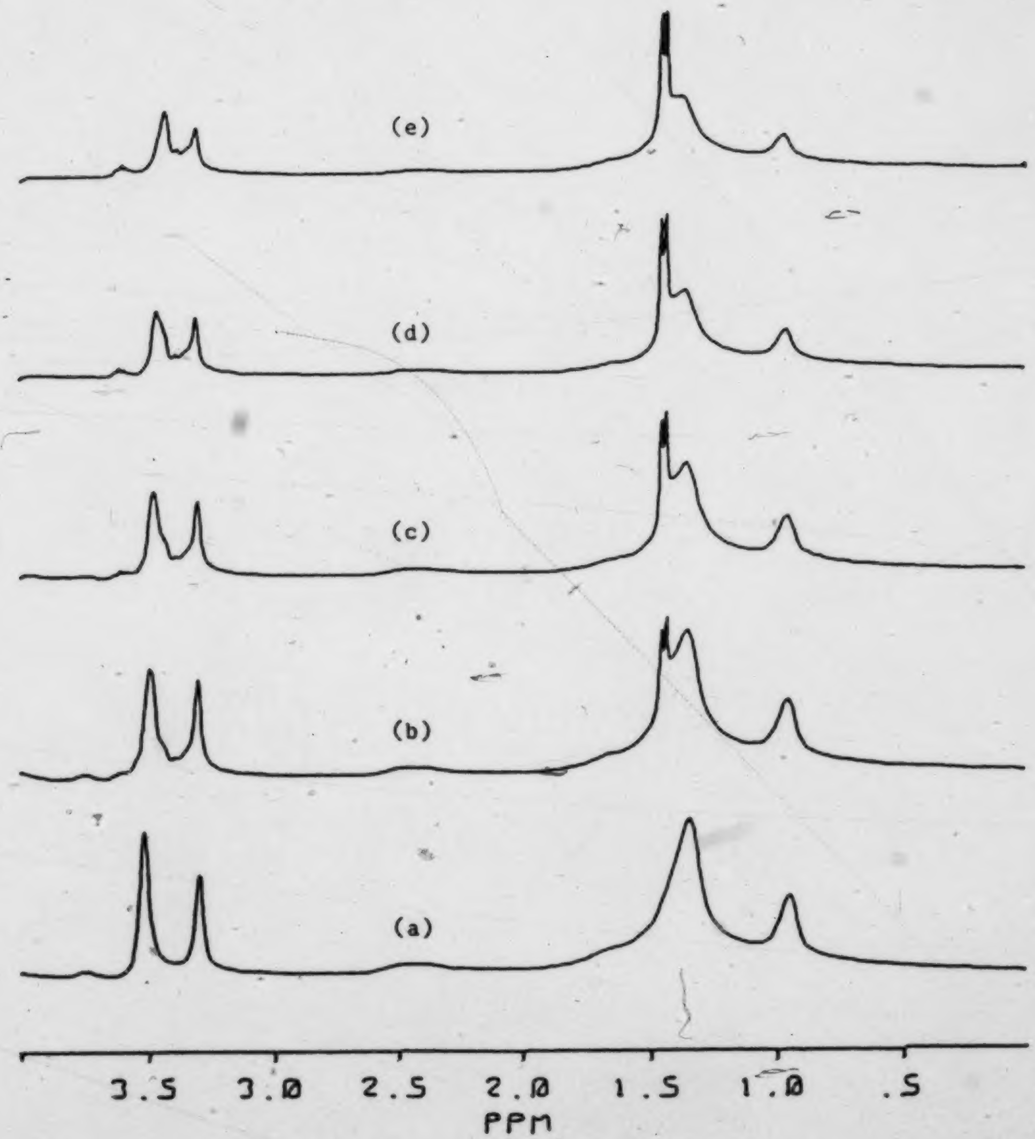

Figure A-5. ¹H-NMR spectra of DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) + OPL : a) 0 mM; b) 5 mM; c) 10 mM; d) 15 mM and e) 20 mM.

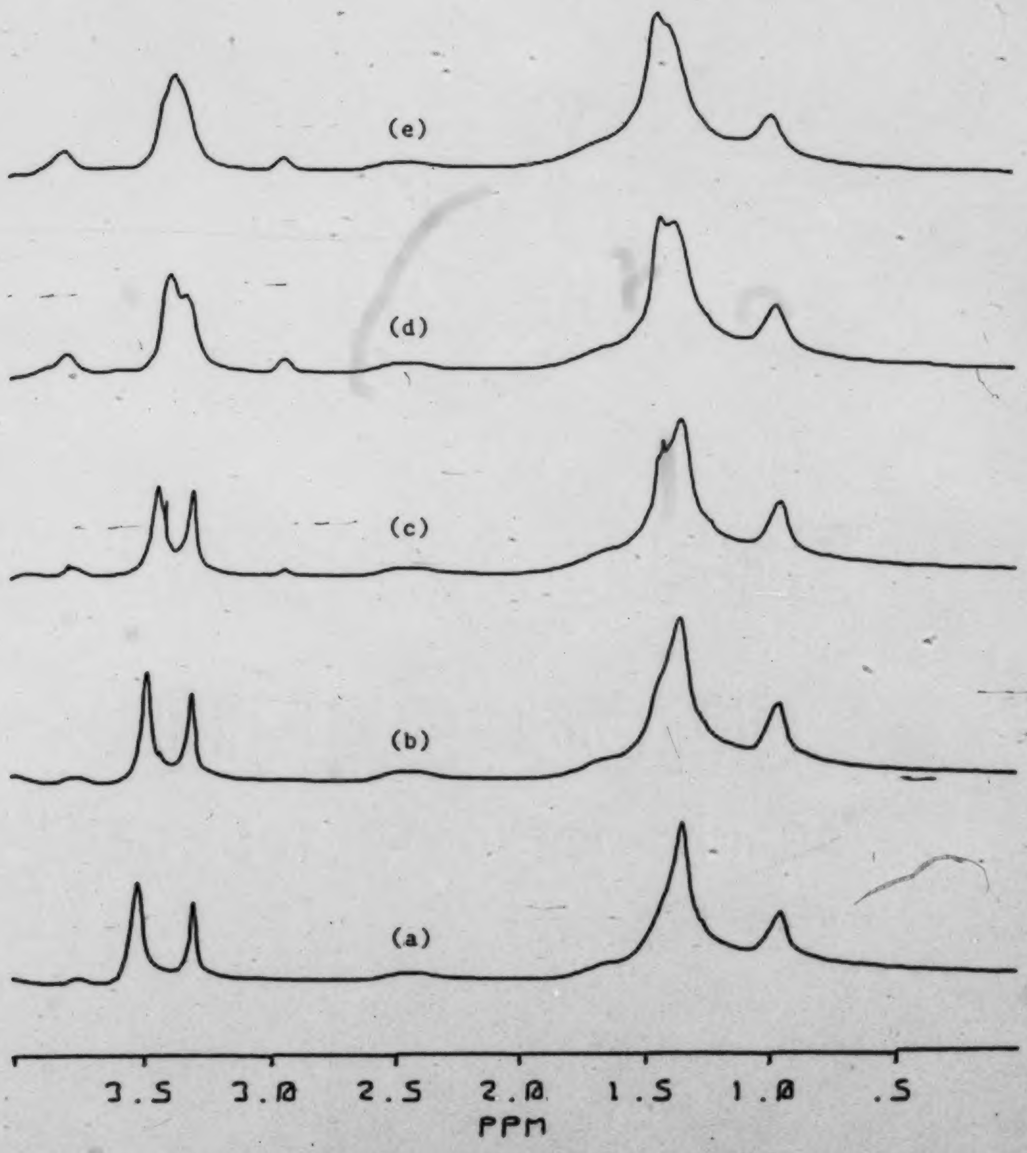

Figure A-6. ¹H-NMR spectra of DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) + MPL : a) 0 mM; b) 1 mM; c) 3 mM; d) 6 mM and e) 8 mM.

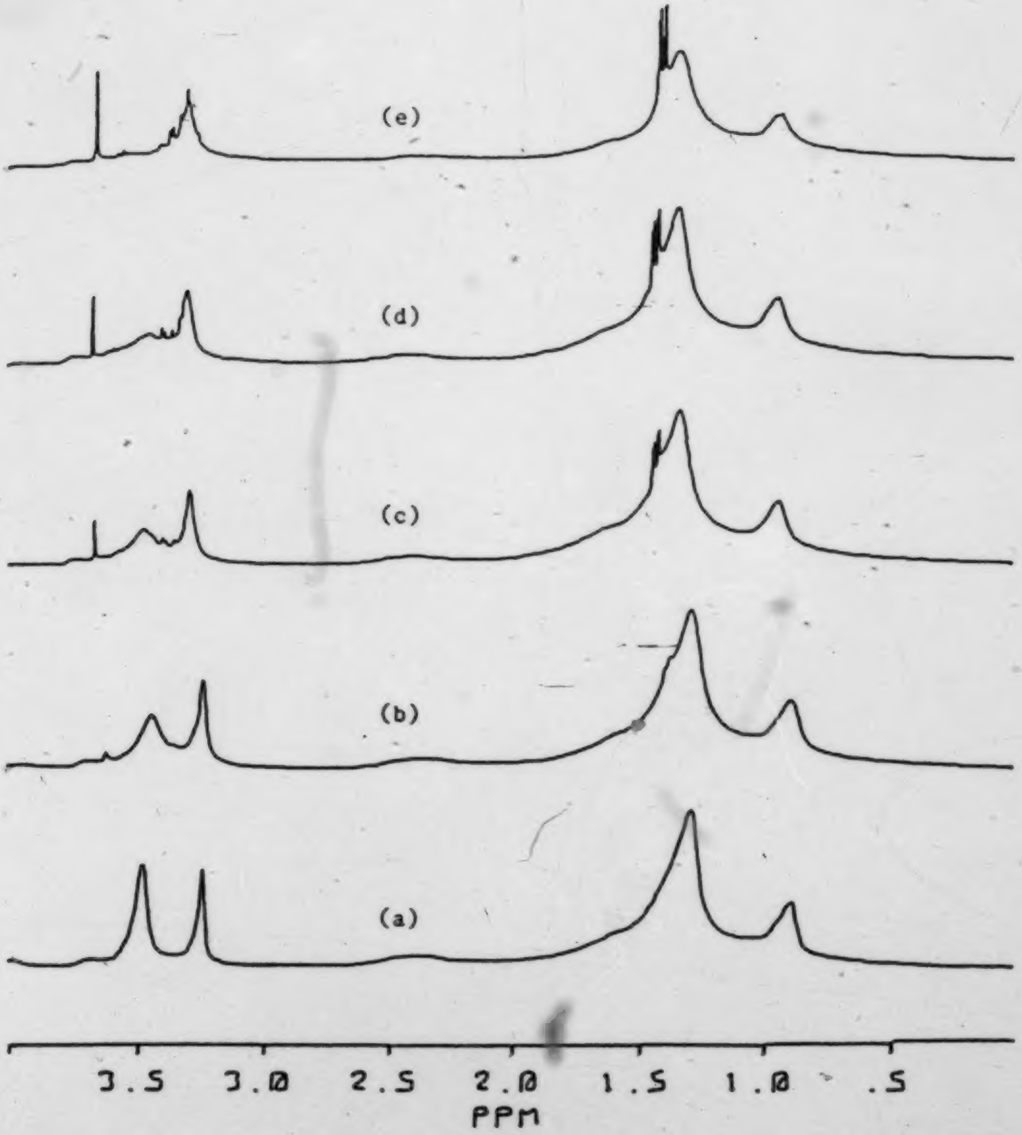

Figure A-7. ¹H-NMR spectra of DMPC(14.4 mM) + $Pr^{3+}$ (2 mM) + ATL : a) 0 mM; b) 1 mM; c) 2 mM; d) 3 mM and e) 5 mM.